THE AMERICAN JOURNAL OF PATHOLOGY

VOLUME XXIX MAY–JUNE, 1953 NUMBER 3

MYOCARDITIS IN ASSOCIATION WITH VARICELLA *

DONALD B. HACKEL, M.D.†

(From the Departments of Pathology, Cleveland City Hospital and Western Reserve University School of Medicine, Cleveland, Ohio)

A number of recent reports have emphasized the relatively frequent occurrence of inflammatory lesions of the myocardium in conjunction with certain infectious diseases, particularly diseases of viral etiology. In 1941 Saphir[1] reviewed the numerous conditions which had been shown previously to be associated with myocarditis, emphasizing its occurrence in such contagious diseases as measles, mumps, and scarlet fever. The following year Saphir and Wile[2] found myocarditis in 6 of 7 patients with poliomyelitis, and in 1949 Ludden and Edwards[3] described the condition in 14 of 35 patients with fatal poliomyelitis. Aschoff was cited by Kirch[4] as finding degenerative and inflammatory changes in the myocardium of patients dying with smallpox. Numerous other recent reports have been concerned with myocarditis in such conditions as influenza,[5] virus pneumonia,[6] and infectious mononucleosis.[6]

In the light of these findings it is not unreasonable to suspect that similar myocardial involvement could occur in other viral and infectious diseases. The present report is concerned with the presence of focal myocardial lesions in 7 cases of chickenpox. As far as can be determined, this is the first description of the occurrence of myocardial lesions in patients suffering from chickenpox.

METHODS

This study was stimulated by the necropsy finding of focal inflammatory lesions in the myocardium of a 32-year-old woman who had a severe case of varicella (case 1). In reviewing the 11,700 necropsies performed at the Cleveland City Hospital between Jan-

* Presented at the Forty-ninth Annual Meeting of the American Association of Pathologists and Bacteriologists, New York City, April 10, 1952.

Received for publication, December 17, 1952.

† This work was done in part during the tenure of a Research Fellowship of the American Heart Association.

uary 1, 1932, and July 1, 1952, 10 additional cases were found in which the patients had died with active varicella. Two of these had an associated diphtheria, and were therefore discarded from this series. Both of these cases, however, did have a marked focal interstitial myocarditis. A third case was discarded, since the patient had *Staphylococcus aureus* pyemia, with abscesses in many organs including the heart. An additional case was excluded because the patient had malignant lymphoma with infiltration of the myocardium by the lymphomatous elements. The remaining 7 cases formed the basis of the study.

The original microscopic slides and paraffin blocks were available for each necropsy. The paraffin blocks of the myocardium were recut and stained with hematoxylin and eosin in all cases, and with eosin and methylene blue and the periodic acid-Schiff's stain in some instances. All organs were available in cases 1 and 7, and the original formalin-fixed tissues were available in 3 of the other 5 cases. Numerous additional sections were taken for microscopic study from all regions of the heart in these cases. Six normal persons of comparable age who died suddenly of traumatic causes were used as a control group, and a comparable number of heart sections were studied in a similar manner.

Clinical Notes

The clinical diagnosis of varicella was unequivocal in all of these cases, and was made on the basis of the typical skin lesions plus a history of recent close exposure. Some of the pertinent details and clinical manifestations correlated with the pathologic findings are summarized in Table I. Six of the patients were children and one was an adult. In no case was myocarditis suspected clinically and in every case there were other conditions that seemed to be adequate as a cause for death.

No chemotherapeutic agents were given in cases 3, 4, and 6. The patient designated as case 1 died after 14 hours of treatment. She was given one (2 gm.) oral dose of sulfadiazine and an intravenous saline drip containing 200,000 units of penicillin and 1 gm. of chloromycetin. This was followed by 100,000 units of penicillin intramuscularly every 2 hours for five times, 500 mg. of chloromycetin orally every 4 hours for two times, and a single intramuscular injection of 1 gm. of streptomycin. The patient designated as case 5 received two intramuscular injections of 20,000 units of penicillin, and in case 7 the patient received a single intravenous dose of 250 mg. of terramycin in 100 cc. of normal saline solution.

TABLE I

Clinical and Pathologic Findings in Seven Patients with Associated Varicella and Myocarditis

Case no.	Age	Sex	Duration of disease	Clinical diagnoses	Pathologic diagnoses†			
					Heart			
					Myoc.	Epic.	Lungs	Other
	yrs.		*days*					
1 (A50-465)*	32	F	5	Varicella, bronchopneumonia, 6 mos. pregnancy	2+	2+	Br.pn. 4+	Chronic passive hyperemia of liver and spleen; skin of fetus uninvolved
2 (A50-144)*	6	M	10	Varicella, purpura due to varicella	1+	±	Int.pn. 1+	Recent hemorrhages: subcutaneous, perinephric, and retroperitoneal; focal necrosis of liver
3 (A8476)*	2	M	6	Varicella, varicella encephalitis	1+	1+	Int.pn. 1+	Cerebral edema
4 (A12032)*	7	F	11	Varicella, ruptured appendix with pelvic abscess	2+	1+	Int.pn. 2+	Pelvic abscess secondary to ruptured appendix; hyperemia of viscera
5 (A16043)*	7	M	3	Varicella, pulmonary edema, hemorrhagic nephritis, endocrine disturbance with precocity	2+	1+	Br.pn. 2+	Lacerations of kidneys with perirenal hemorrhage; adrenal hyperplasia; adrenal rests in testes; focal necrosis of spleen
6 (A12164)*	1/6	F	3	Varicella, bronchopneumonia	1+	2+	Br.pn. 2+	
7 (A52-165)*	2	M	6	Varicella, ruptured appendix with generalized peritonitis, cardiac arrest (during surgery)	1+	0	Edema and hyperemia	Diffuse peritonitis secondary to ruptured appendix

* Cleveland City Hospital necropsy number.

† Estimate of severity of lesions: 0 = none; ± = very slight; 1+ = slight; 2+ = moderate; 4+ = marked.
Br.pn. = bronchopneumonia; Int.pn. = interstitial pneumonitis; Epic. = epicarditis; Myoc. = myocarditis.

Pathologic Findings

Heart. There were no gross abnormalities in the heart, except for small epicardial petechiae in cases 2, 4, and 5. Microscopic examination revealed scattered focal lesions in all cases. In all cases except no. 1, however, the heart was originally considered to be normal on the basis of one or two sections. Re-examination of these hearts revealed the lesions which will be described. In 4 cases (nos. 2, 3, 6, and 7) these lesions were considered to be slight, showing a moderate amount of interstitial edema and small focal collections of mononuclear cells, lymphocytes and occasional plasma cells, neutrophils, and eosinophils. The infiltrates were often perivascular and extended for a short distance between the muscle fibers. The inflammatory foci were limited in extent, as seen by the fact that serial sections, cut at 6 μ, often showed such foci extending through only two or three levels. The other 3 cases showed a moderate degree of myocarditis. In these the lesions were similar to those labelled as "slight," but showed a much greater degree of leukocytic infiltration. The muscle fibers were uninvolved, for the most part, but they occasionally (cases 1 and 6) showed evidence of necrosis, with globular swollen ends that were deeply eosinophilic without cross striations. In none of the cases were any inclusion bodies observed. There was a slight to moderate degree of epicarditis in all cases but no. 7, with scattered collections of lymphocytes, mononuclear cells, and occasional plasma cells.

Lungs. There were no inflammatory changes in the lungs in case 7. All other cases showed some pneumonia. Case 1 had a marked bronchopneumonia. Microscopic examination showed much edema fluid with fibrin strands occasionally forming hyaline membranes in the alveoli, together with an inflammatory exudate consisting mainly of mononuclear cells. There were also scattered small abscesses, with marked acute inflammation and necrosis. Cases 5 and 6 showed a moderate bronchopneumonia, with some edema and intra-alveolar exudate of fibrin, large mononuclear cells, lymphocytes, and neutrophils. Cases 2, 3, and 4 showed a slight to moderate interstitial pneumonitis, with neutrophils and lymphocytes in the alveolar walls and some edema fluid and a few mononuclear cells within the alveoli. No inclusion bodies were observed in any of these sections.

Miscellaneous Organs. There was no evidence of cardiac failure in any case, although cases 1 and 3 showed some visceral hyperemia. The marked hemorrhagic tendency in case 2 was unexplained, since bleeding and clotting times were normal. The hemorrhages in case 5

also were not explained, and no history of trauma was given. The spleen in case 5 was enlarged, weighing 200 gm., and it contained several small foci of necrosis. The liver in case 2 had a similar small focus of acute inflammation and necrosis. Post-mortem cultures of the heart's blood were taken in all cases but case 4, and yielded no organisms. Lung culture in case 2 gave no growth; in case 1, *Staphylococcus albus;* in case 6, a mixed flora including unidentified streptococci; in case 7, hemolytic *Staphylococcus aureus* (probably a contaminant), and alpha streptococcus.

Control Cases. The hearts of 6 persons who died suddenly of traumatic causes showed no changes that resembled the myocardial lesions in the 7 patients dying with chickenpox. One of the 6 "normal" hearts contained a single small focus of about a dozen lymphocytes between muscle fibers. There was no edema and the myocardial fibers were not distorted. A heart from the oldest patient in the series, a 32-year-old man, had a moderate amount of perivascular fibrosis. There were also a few regions in this case in which the muscle fibers were separated by what seemed to be a small amount of edema fluid. However, there was no inflammatory infiltrate present.

Discussion

Since definite myocardial lesions were found in all 7 of the cases examined, it is surprising that no previous observations have been reported of the association of chickenpox and myocarditis. In a review of myocarditis in children, Saphir, Wile, and Reingold[7] stated that "Judging from the lack of available literature, myocarditis in other contagious diseases in children, such as smallpox and varicella, seems to be exceedingly rare. However, one must take into consideration that there probably are no studies available with detailed microscopic examinations of the heart. Such studies are needed to prove whether or not the concept of the rarity of myocarditis in these diseases is correct." Fatal chickenpox is so unusual that very few cases are seen by any one pathologist. Most of the previous necropsy reports have been concerned with the finding of a severe pneumonitis,[8] encephalitis,[9] or other concomitant findings such as thrombocytopenic purpura.[10] There is no clinical indication of myocardial involvement and attention is therefore probably diverted from the heart, which is examined by only a routine number of microscopic sections. In this way it is easy to overlook the local lesions which have been described.

In none of the cases included in this study were there any significant clinical signs of cardiac involvement, nor was there any post-

mortem evidence of cardiac failure. Furthermore, in every case there were other significant findings to account for death. It is therefore believed that the focal myocardial lesions were incidental findings, which would have healed unnoticed had not death from other causes permitted their discovery. Patients with varicella or other diseases, such as measles and mumps, which have been previously shown to produce myocardial lesions of similar character, may thus develop slight focal inflammatory lesions in the myocardium which are never severe enough to produce clinical manifestations. Healing of these lesions might leave small fibrotic foci which could account for the frequent necropsy observation of focal myocardial fibrosis in patients with no history of previous rheumatic fever or other cardiac involvement. It might also account for the otherwise unexplained clinical finding of so-called benign bundle branch block, as pointed out by Vazifdar and Levine.[11]

The myocardial lesions in these 7 patients with chickenpox are similar to those which have been attributed to sulfonamide therapy.[6,12] However, only one patient (case 1) in the present series received such therapy, which consisted of merely a single 2 gm. dose of sulfadiazine.

In all cases but one there was some bronchopneumonia or interstitial pneumonitis. Previous studies have pointed out the occurrence of myocarditis in patients in whom the main necropsy finding was pneumonia. Stone[13] found myocardial lesions in one of 37 patients with bronchopneumonia, and Gore and Saphir[6] found myocarditis in 32 of 222 patients with virus pneumonia. However, considering the 100 per cent incidence of myocarditis in the present series, it seems likely that the myocardial involvement is due to some factor other than the pneumonia. It is possible that varicella virus is the actual etiologic agent, but this cannot be considered as established without isolation of the virus from the myocardium and its use in the experimental production of similar lesions.

Certain other features in some of these 6 cases deserve brief comment. A bleeding tendency was present in cases 2 and 5. The simultaneous occurrence of chickenpox and thrombocytopenic purpura has been noted[10] but no evidence for thrombocytopenia existed in these 2 cases. Johnson[14] observed visceral hemorrhages related to capillary destruction and thrombosis, as did Waring, Neubuerger and Geever.[9] No vascular lesions were observed in any of the cases in the present series.

A small focus of necrosis was present in the liver in case 2 and several necrotic foci were present in the spleen in case 5. Similar lesions

in cases of varicella have been described by Schleussing,[15] Oppenheimer,[16] and Lucchesi, La Boccetta, and Peale.[17]

Waring *et al.*[9] described an "acute toxic encephalitis" in a patient dying with chickenpox. In their case there were petechiae of the white matter, slight round cell cuffing of blood vessels, and focal microglial proliferation. In case 3, in which the clinical diagnosis of encephalitis was made, the only related necropsy finding was cerebral edema.

Summary

Focal interstitial inflammatory lesions were observed in the hearts of 7 patients who had varicella at the time of death.

Examination of the hearts of 6 healthy people who died suddenly of traumatic causes revealed no similar lesions.

It is suggested that myocardial lesions might occur without clinical manifestation in non-fatal cases of chickenpox and other virus diseases and could account for the frequent necropsy observation of focal myocardial fibrosis in patients with no history of previous rheumatic fever or other cardiac disease.

The hearts of the 6 "normal" controls were obtained through the kind cooperation of Dr. Lester Adelson, pathologist to the Coroner of Cuyahoga County.

REFERENCES

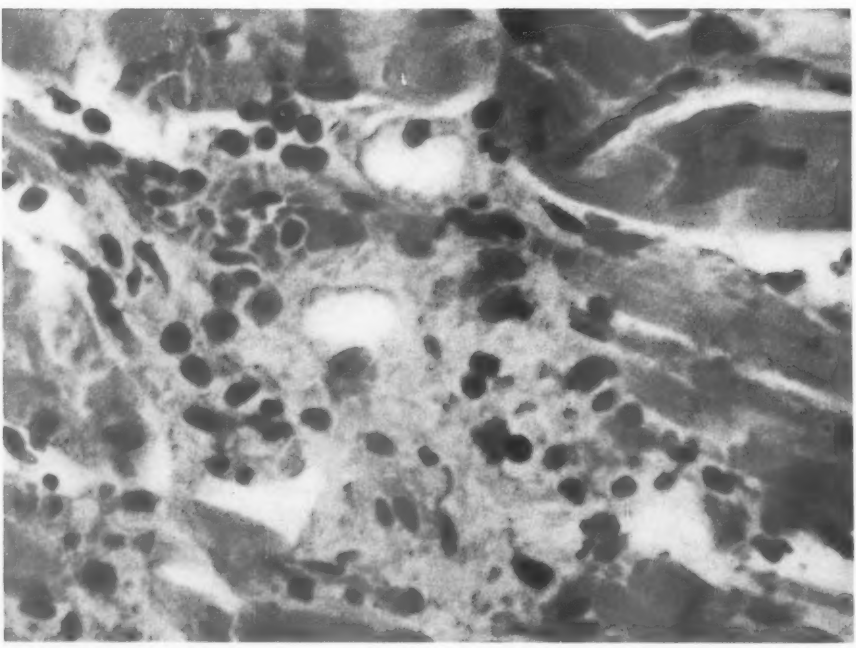
1. Saphir, O. Myocarditis; a general review with an analysis of two hundred and forty cases. *Arch. Path.*, 1941, **32**, 1000–1051. *Ibid.*, 1942, **33**, 88–137.
2. Saphir, O., and Wile, S. A. Myocarditis in poliomyelitis. *Am. J. M. Sc.*, 1942, **203**, 781–788.
3. Ludden, T. E., and Edwards, J. E. Carditis in poliomyelitis. An anatomic study of thirty-five cases and review of the literature. *Am. J. Path.*, 1949, **25**, 357–381.
4. Kirch, E. Pathologie des Herzens. *Ergebn. d. allg. Path. u. path. Anat.*, 1927, **22**, 1–206.
5. Finland, M., Parker, F., Jr., Barnes, M. W., and Joliffe, L. S. Acute myocarditis in influenza A infections. Two cases of nonbacterial myocarditis, with isolation of virus from the lungs. *Am. J. M. Sc.*, 1945, **209**, 455–468.
6. Gore, I., and Saphir, O. Myocarditis. A classification of 1402 cases. *Am. Heart J.*, 1947, **34**, 827–830.
7. Saphir, O., Wile, S. A., and Reingold, I. M. Myocarditis in children. *Am. J. Dis. Child.*, 1944, **67**, 294–312.
8. Frank, L. Varicella pneumonitis. Report of a case with autopsy observations. *Arch. Path.*, 1950, **50**, 450–456.
9. Waring, J. J., Neubuerger, K., and Geever, E. F. Severe forms of chickenpox in adults. With autopsy observations in a case with associated pneumonia and encephalitis. *Arch. Int. Med.*, 1942, **69**, 384–408.
10. Cohen, J. J., and Bansmer, C. Chickenpox with simultaneous idiopathic thrombocytopenic purpura. Report of a case. *New England J. Med.*, 1947, **237**, 222–224.
11. Vazifdar, J. P., and Levine, S. A. Benign bundle branch block. *A. M. A. Arch. Int. Med.*, 1952, **89**, 568–574.

12. French, A. J., and Weller, C. V. Interstitial myocarditis following the clinical and experimental use of sulfonamide drugs. *Am. J. Path.*, 1942, **18**, 109–121.
13. Stone, W. J. The heart muscle changes in pneumonia, with remarks on digitalis therapy. *Am. J. M. Sc.*, 1922, **163**, 659–668.
14. Johnson, H. N. Visceral lesions associated with varicella. *Arch. Path.*, 1940, **30**, 292–307.
15. Schleussing, H. Nekrosen in Leber, Milz und Nebennieren bei nicht vereiterten Varizellen. *Verhandl. d. deutsch. path. Gesellsch.*, 1927, **22**, 288–292.
16. Oppenheimer, E. H. Congenital chickenpox with disseminated visceral lesions. *Bull. Johns Hopkins Hosp.*, 1944, **74**, 240–250.
17. Lucchesi, P. F., La Boccetta, A. C., and Peale, A. R. Varicella neonatorum. *Am. J. Dis. Child.*, 1947, **73**, 44–54.

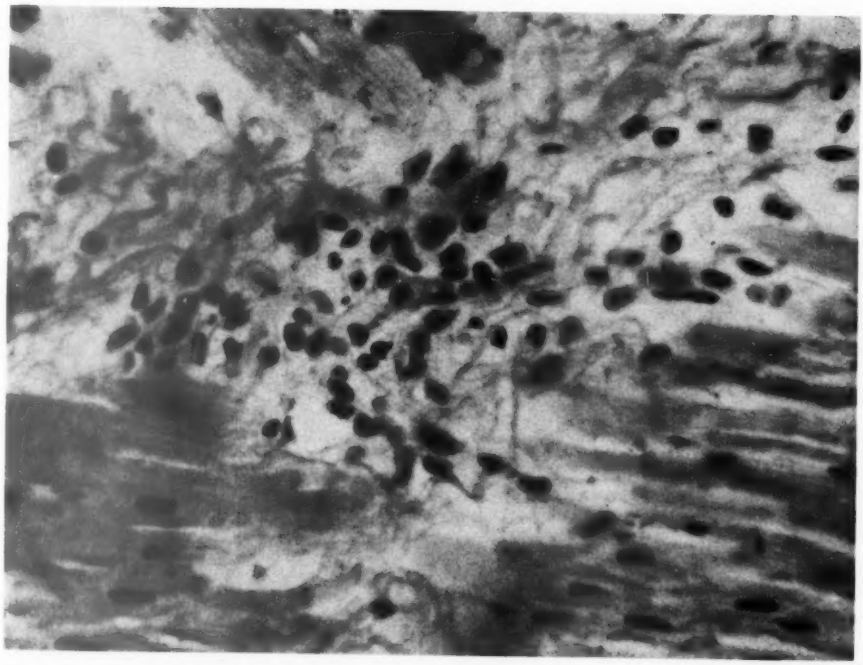
LEGEND FOR FIGURES

FIGS. 1 to 7. These illustrations are from cases 1 to 7, respectively, and illustrate varying degrees of interstitial edema and of infiltration by large mononuclear cells, lymphocytes, plasma cells, and polymorphonuclear leukocytes. Hematoxylin and eosin stain. × 540.

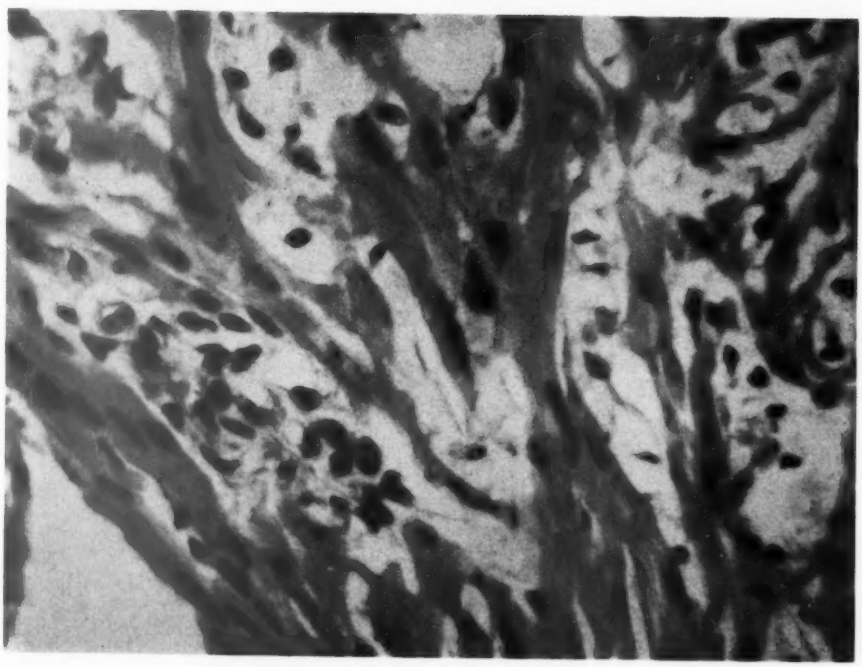
1



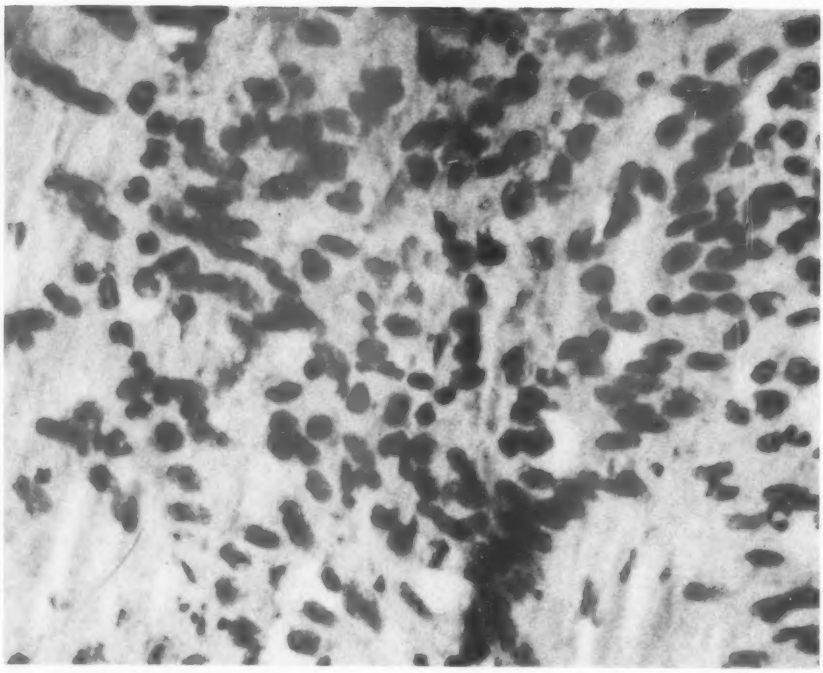
2



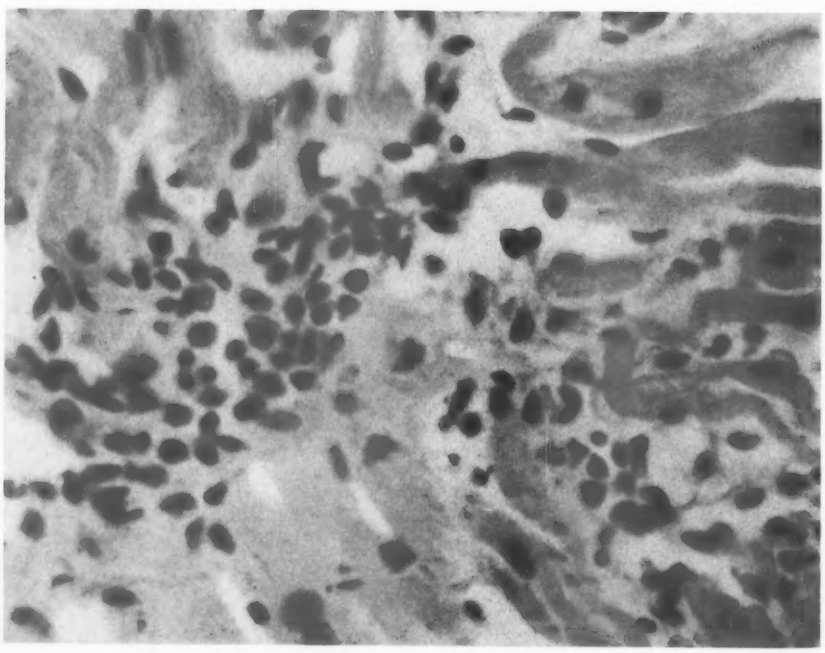
3



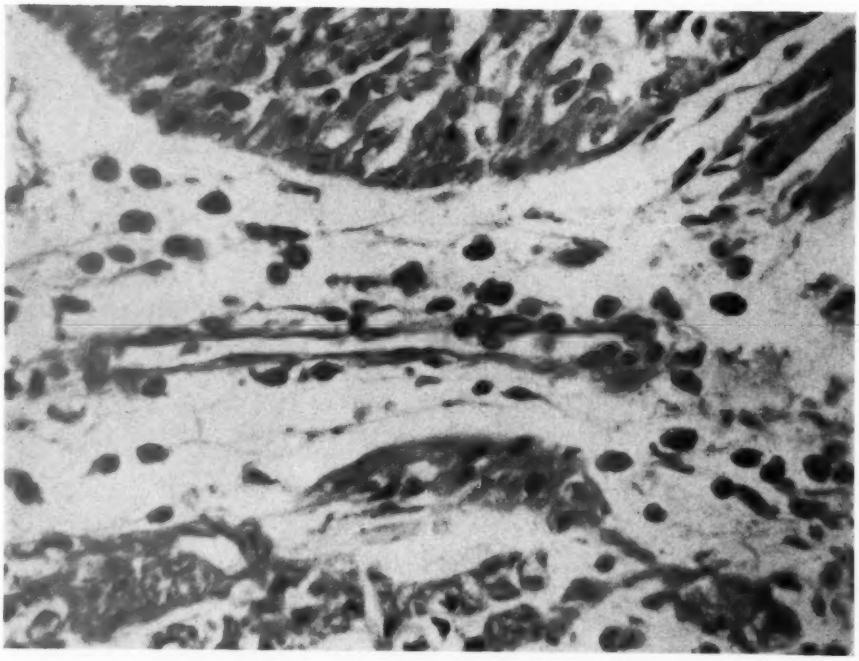
4



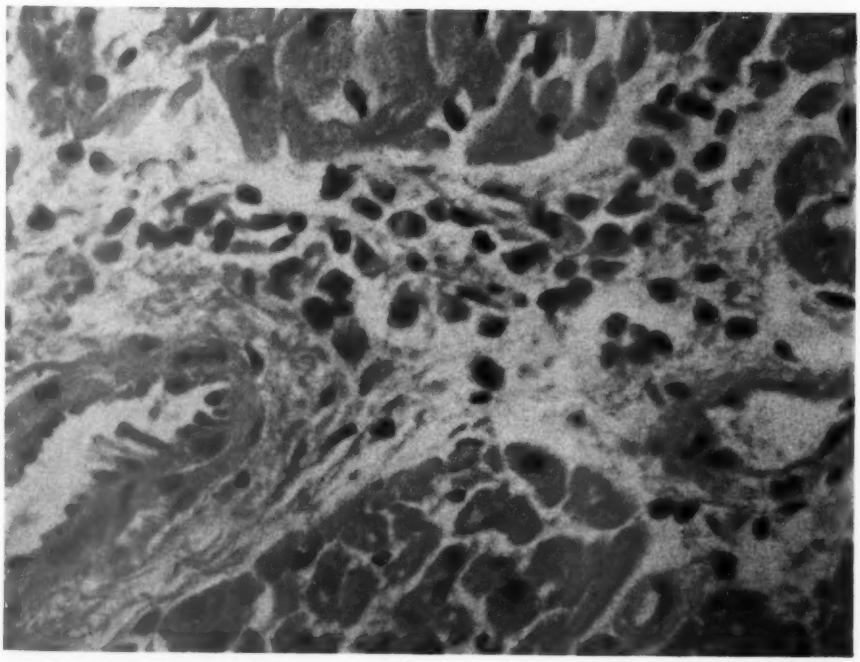
5



6



7



HISTOPATHOGENESIS OF CORTISONE-ALTERED EXPERIMENTAL POLIOMYELITIS

Observations on the Syrian Hamster Inoculated Intracerebrally with Strain MEF_1*

Stanley M. Aronson, M.D., and Gregory Shwartzman, M.D.
(*From the Department of Microbiology, the Mount Sinai Hospital, New York 29, N.Y.*)

Previous observations demonstrated that cortisone markedly enhances poliomyelitis infection in the Syrian hamster.[1,2] The potentiation is evident whether the viral inoculum is given by the intracerebral or parenteral route.[3] In the absence of cortisone there is seen only a mild disease with a low mortality rate following intracerebral inoculation and no apparent infection following parenteral inoculation of the virus. Since cortisone is capable of inhibiting the reaction of tissues to noxious agents,[4-6] the following detailed morphologic study of cortisone-enhanced poliomyelitis was deemed desirable.

Methods and Materials

Strain MEF_1 was kindly supplied by Dr. Peter K. Olitsky and maintained in this laboratory by serial intracerebral mouse passages. An emulsion of a large number of mouse brains preserved in an electric refrigerator at −30° C. was used for these experiments. The LD_{50} mouse titer was $10^{-8.7}$. A neutralization test for the control identification of the virus was carried out with an anti-Lansing monkey convalescent serum. Syrian male hamsters weighing 20 to 28 gm. were employed for the experiment.

When needed for pathologic studies, the animals were killed with chloroform and necropsied immediately after cessation of respiration. All tissues were preserved in 10 per cent formalin solution. Brains were sectioned serially at 6 μ and slides were stained alternately with hematoxylin and eosin, and thionine (Nissl stain). The osseous structures encasing the spinal cord and other soft structures were decalcified in 5 per cent formic acid and sectioned at appropriate levels. Sections of spinal cord were stained with hematoxylin and eosin, thionine, Mahon's stain for myelin,[7] and Mallory's phosphotungstic acid-hematoxylin. Sympathetic ganglia were isolated with the aid of a dissecting microscope, embedded, sectioned, and stained by these

* Aided by a grant from the National Foundation for Infantile Paralysis, Inc.
Presented at the Forty-ninth Annual Meeting of the American Association of Pathologists and Bacteriologists, New York City, April 10, 1952.
Received for publication, October 25, 1952.

methods. Viscera and peripheral nerves were sectioned, stained with hematoxylin and eosion, and, where applicable, with thionine, Mahon's, Giemsa's, scharlach R (fat), Gallego's trichrome,[8] and von Kossa's stains.

Results

Clinical Course of Poliomyelitis Infection in Cortisone-Treated Hamsters Following Intracerebral Inoculation of MEF_1

In order to permit concurrent clinical and pathologic studies, animals treated similarly to those used for sectioning were observed daily for a period of 1 month. The course of the disease is recorded in Table I.

Table I

Effect of Cortisone upon the Clinical Course of Poliomyelitis Infection in the Hamster

Group	No.	Number paralyzed	Percentage of paralytic animals showing bilateral involvement	Percentage of paralytic animals showing tetraplegia	Number dying without paralysis	Percentage of mortality in paralytic group	Average onset of paralysis	Interval between paralysis and death
A	20	19	84%	35%	1	100%	5.0 days	2.2 days
B	20	10	60%	15%	0	60%	5.2 days	4.0 days

Animals in groups A and B were inoculated intracerebrally with 0.05 ml. of MEF_1 diluted 1:20 (mouse pool III).

Animals of group A also were given intramuscularly 5 mg. of cortisone simultaneously with the virus.

The clinical observations were essentially in accord with those previously published.[1] In the absence of cortisone about 50 per cent of hamsters gave no clinical evidence of paralytic disease. In the presence of cortisone the ensuing disease was more fulminant, showed a greater number of limbs involved, a greater tendency toward bilateral extension, and a comparatively higher mortality rate.

Pathologic Studies on Poliomyelitis Infection in Cortisone-Treated Hamsters Following Intracerebral Inoculation of MEF_1

One hundred hamsters were subdivided into control and experimental groups as shown in Table II. Two or more hamsters from each group were sacrificed 18 hours after the intracerebral inoculation and daily thereafter.

Central Nervous System

In hamsters receiving the virus intracerebrally and cortisone intramuscularly (group A, Tables II and III) a diagonal needle tract usually traversed the dorsal neocortex and almost invariably extended

ventro-inferiorly through the right hippocampal cortex and right lateral neocortex. The neighboring neuronal planes were disrupted and hemorrhagic but no inflammatory or interstitial reaction was visible. The first day following inoculation, the hemorrhagic needle tract showed no inflammatory, vascular, or parenchymal reaction. No neuronal lesions were present other than those resulting from injury in inoculation. No significant resorption of the hemorrhage in the needle tract was seen in the animals sacrificed after 2 days. Neurons of the

TABLE II
Experimental Conditions in Five Groups of Hamsters

Group	No. of hamsters	Intracerebral inoculum	Cortisone
A	40	0.05 cc. MEF1 stock mouse passage diluted 1:20	5 mg., intramuscularly, simultaneously with intracerebral inoculation
B	40	0.05 cc. MEF1 stock mouse passage diluted 1:20	None
C	5	0.05 cc. sterile mouse brain emulsion, diluted 1:20	5 mg., intramuscularly, simultaneously with intracerebral inoculation
D	5	0.05 cc. sterile mouse brain emulsion, diluted 1:20	None
E	10	None	5 mg., intramuscularly

ipsilateral hippocampal and dentate gyri revealed early degeneration, consisting of peripheral chromatolysis, nuclear pyknosis, shrinkage, and distortion. There was no accompanying inflammatory cell congregation or vascular reaction.

After 3 days the needle tract still showed no attempts at repair. Much of the hemorrhage was retained although some hemosiderin-like pigment was seen lying free in the traumatic defect. Almost all neurons of the inferior loop of the ipsilateral hippocampal gyrus were either missing or reduced to pale, eosinophilic, anuclear spheres. The involved zones were conspicuously free of inflammation, microglial response, or vascular activation. A similar locus of neuronal loss was apparent in the contralateral hippocampus. An unusual feature was the precise symmetry of involvement. The ipsilateral tuberculum olfactorium presented a moderate loss of cortical neurons, with only a linear aggregation of empty spaces to indicate their former location. Degenerative changes in neurons of the lumbar anterior horn were seen in one cortisone-treated hamster sacrificed on the third day.

Sections taken from 4-day animals revealed no evidence of repara-

tion of the principal segments of the needle tract (Fig. 2). The fragmented bordering parenchyma was devoid of inflammatory cells or microglial concentration. Some free hemosiderin was seen, but fresh hemorrhage still separated the apposing wound surfaces. Occasionally there was a feeble inflammatory response at the termination of the needle tract. In one animal, a single neighboring vessel was ringed with round cells. The necrobiotic changes in the hippocampal and

TABLE III

Tabulation of Extent and Degree of Severity of Lesions Within Central Nervous System

Day		1		2		3		4		6		7		8		9		10	
Groups		A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
Site																			
Olfactory bulb	L.	0*	0	0	0	0	0	0	0	0	0	0	0	3	0	3	0	–†	0
	R.	0	0	0	0	0	0	0	0	3	0	0	0	3	0	1	0	–	0
Olfactory tubercle	L.	0	0	0	0	0	0	0	0	2	0	2	0	4	0	3	0	–	0
	R.	0	0	0	0	2	0	1	0	2	0	2	0	4	0	3	0	–	0
Septum		0	0	0	0	0	0	0	1	0	0	1	0	0	0	1	0	–	0
Neocortex	L.	0	0	0	0	0	0	0	0	0	1	0	1	0	1	2	0	–	0
	R.	0	0	0	0	0	1	2	1	2	2	2	0	2	0	2	1	–	0
Hippocampal cortex	L.	0	0	0	0	1	1	2	0	3	0	4	1	3	1	3	1	–	2
	R.	0	0	2	0	2	1	2	0	3	1	4	1	3	1	3	1	–	0
Dentate gyrus	L.	0	0	0	0	0	0	0	0	1	0	1	0	1	0	2	0	–	0
	R.	0	0	2	0	0	0	2	0	1	0	2	0	1	0	2	0	–	0
Amygdaloid nuclei	L.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0	–	0
	R.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	–	0
Basal ganglia	L.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	–	0
	R.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	–	0
Midbrain	L.	0	0	0	0	0	0	0	0	1	1	0	1	0	0	2	0	–	0
	R.	0	0	0	0	0	2	0	0	1	1	1	1	1	1	1	0	–	0
Third nerve nucleus	L.	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	–	0
	R.	0	0	0	0	0	1	0	0	1	0	2	0	0	0	0	0	–	0
Fifth nerve nucleus	L.	0	0	0	0	0	0	0	0	2	2	2	0	2	2	0	0	–	0
	R.	0	0	0	0	0	0	0	0	2	2	2	3	0	2	0	0	–	0
Pons	L.	0	0	0	0	0	0	0	0	2	0	1	0	0	0	0	0	–	0
	R.	0	0	0	0	0	0	0	0	2	1	1	0	0	0	0	0	–	0
Nuclei of medulla	L.	0	0	0	0	0	0	0	0	2	0	1	0	2	2	0	0	–	0
	R.	0	0	0	0	0	0	0	0	0	0	2	0	2	2	0	0	–	0
Reticular formation	L.	0	0	0	0	0	0	0	0	2	0	2	1	2	1	1	0	–	0
	R.	0	0	0	0	0	0	0	0	2	2	2	1	2	1	1	0	–	0
Cervical cord	L.	0	0	0	0	0	0	1	0	2	2	2	0	4	4	1	0	–	0
	R.	0	0	0	0	0	0	1	0	2	2	2	0	4	4	2	0	–	0
Thoracic cord	L.	0	0	0	0	0	0	0	0	1	0	1	0	2	2	2	0	–	0
	R.	0	0	0	0	0	0	0	0	1	0	1	0	2	2	1	0	–	0
Lumbar cord	L.	0	0	0	0	1	0	0	0	1	0	1	0	4	3	1	0	–	0
	R.	0	0	0	0	1	0	0	0	1	0	1	0	3	3	0	0	–	0
Meningitis‡		0	2	0	1	0	2	0	2	1	3	0	3	0	1	1	1	–	0

* Numbers express degree of neuronal destruction, 4 being most severe.

† No animals in group A survived 10 days.

‡ Numbers herein represent degree of meningeal exudate, 4 being most severe.

Animals in groups A and B were inoculated intracerebrally with 0.05 ml. of MEF_1 diluted 1:20 (mouse pool III).

Animals in group A also were given intramuscularly 5 mg. of cortisone simultaneously with the virus.

dentate gyri, seen on the second and third days, were again visible. Circumscribed zones of total neurocytolysis were also apparent in the neocortex and pyriform cortex. Except for the one perivascular response in the hippocampal cortex already described, the vessels supplying the areas of cortical degeneration were neither congested, hyperplastic, nor surrounded by inflammatory elements. In one animal of this group, a few granulocytes occupied the tissue defects secondary to hippocampal neuronolysis. Some slight microglial concentration in this region was also apparent. The anterior horn cells of the cervical cord were often minimally distorted, sclerotic, and reduced in number. There was no tissue reaction to this change.

By the sixth day, a number of phagocytic cells, some containing hemosiderin, were concentrated along the needle tract. There were bilaterally extensive zones of neuronal loss in the hippocampal gyri with only a line of empty tissue spaces remaining. No inflammatory or vascular response was detectable. Rostrally, the tuberculum olfactorium showed bilateral neurocytosis. Still further anteriorly, regions of necrobiosis were perceptible, involving the mitral layer of the ipsilateral olfactory bulb. Within the brain stem, lesions were seen in substantia nigra, ipsilateral oculomotor, trigeminal, interpeduncular, solitarius, vestibular, and abducens nuclei. These destructive changes were but rarely attended by supervening inflammatory or microglial reaction. Below the midbrain these lesions, while bilateral, were more prominent on the contralateral side. The severest brain stem damage was noted in the medullary reticular formation. Widespread destructive changes in anterior horn neurons were demonstrable at all spinal levels. This neurocytolysis was but rarely associated with any cellular reaction. The animals sacrificed on this day showed early clinical paralysis.

Histologic sections derived from animals sacrificed on the seventh, eighth, and ninth days showed essentially the same features as have been described (Figs. 5 and 9). There was, however, a progressive prominence of such reactive features as endothelial hyperplasia, perivascular infiltration, and neuronophagia in levels of the central nervous system remote from the viral depot. The incremented reactivity was particularly striking in sections of the olfactory lobe of animals sacrificed on the eighth and ninth days, in which there was widespread tissue necrosis, with profuse inflammatory exudate extending beyond the confines of olfactory lobe parenchyma and into the perineural spaces about the converging olfactory nerves (Fig. 8). A mild meningitis, of basilar distribution, was noted in brains removed after 9 days.

No demyelinization or glial scarring was demonstrable within the time limits of the experiment. Sections of sympathetic ganglia uniformly failed to demonstrate any histologic abnormalities.

In the animals receiving intracerebral virus and no cortisone (group B, Tables II and III), the diagonal hemorrhagic needle tract, following a course as described previously, was lined by a sparse granulocytic infiltration, 18 hours after intracerebral inoculation. In a few animals the lateral choroid plexus adjacent to the needle tract was characterized by congestion and extravasated masses of neutrophils. Parenchymal vessels in the vicinity of the needle tract often presented prominence of endothelial elements. An acute basilar meningitis extended posteriorly to the level of the cerebellopontine angle. On the second day, a narrow zone of inflammatory cells and phagocytes filled the needle tract. The hemorrhage was considerably resolved. There was an intense round cell infiltration of the traversed parenchyma. Meningeal vessels near the site of inoculation were hyperplastic. Numerous early perivascular cuffs were apparent in the region of injection. The depot site became converted to a mass of vacuolated tissue phagocytes, interspersed with lymphocytes and neutrophils. By the third day, the needle tract was devoid of hemorrhage and filled with reactive elements. An occasional hippocampal neuron showed chromatolysis. The white matter interstitium of the cortex and midbrain showed a pleomorphic inflammatory infiltration and an extensive meningitis was seen. Serial sections disclosed a continuity of meningeal exudate and perivascular cuffing. In the midbrain a few neurons of the oculomotor nucleus were chromatolytic and surrounded by satellite cells. The endothelial lining of the lateral meningeal vessels was often converted to a stratified zone of prominent cells (Fig. 3). The needle tract was discernible by the fourth day as a linear cellular zone composed of "foam cells" and newly formed capillaries (Figs. 1 and 4). While scattered perivascular cuffs were visible throughout the neocortex, no significant neuronal changes were apparent. Neuronal lesions of a minimal nature were visible in sections of the hippocampus, brain stem, and cord following 6 days of viral infection. In most animals, however, lesions were limited to the hippocampal cortex and rostral midbrain. In all instances, there was a florid inflammatory and microglial response (Fig. 6). One animal, sacrificed on the eighth day, showed extensive changes in the spinal anterior horns, reflecting a clinical paralysis (Fig. 10).

In animals receiving sterile mouse brain emulsion intracerebrally and cortisone intramuscularly (group C, Table II), all pathologic

changes were limited to the needle tract and inoculation depot. The tract was hemorrhagic and the traumatized surfaces non-reactive until the fourth day following intracerebral injection. A few rare granulocytes and an increased number of rod cells appeared by this time. Blood vessels were totally quiescent until the sixth day.

In animals receiving sterile mouse brain emulsion intracerebrally and no cortisone (group D, Table II), a prompt reaction was seen to the intracerebral trauma. Animals sacrificed 18 hours after injection already demonstrated neighboring vascular engorgement, increased numbers of granulocytes, and parenchymal edema. The major volume of hemorrhage was absorbed by the fourth day and the rapidly accumulated masses of phagocytes contained granular inclusions of hemosiderin. Tissue continuity was restored by the sixth day and only a linear congregation of reactive cells revealed the needle tract.

In animals receiving cortisone alone (group E, Table III), no intracerebral or spinal changes were apparent in any of those sacrificed.

Viscera

In animals receiving cortisone (groups A, C, and E, Table II), there was noted grossly a progressive diminution in splenic and thymic volume. By the fourth day following cortisone injection, the liver assumed a waxy hue. Bulk of skeletal musculature was perceptibly lessened.

Microscopically, organs containing lymphoid tissue (*i.e.*, spleen, lymph nodes, and thymus) showed a considerable loss of these elements following cortisone administration. The cross-sectional diameter of skeletal muscles was often decreased, presenting the picture of early atrophy. The described changes were visible in all animals receiving cortisone with or without virus.

In hamsters receiving virus in addition to cortisone, two additional somatic changes were demonstrable. The first of these was found in the paravertebral and periadrenal adipose tissue. The microscopic picture consisted of smudginess of the vacuoles and the appearance of minute discrete basophilic bodies lining the inner cell membrane. These granules proceeded rapidly to calcification. The interstices showed the presence of small pleomorphic cells with moderately abundant basophilic cytoplasm. These elements were interpreted as lipoblasts. Still later, inflammation developed. The steatitis first appeared on the sixth day. The second lesion noted was in skeletal musculature, again limited to the vertebral region. The earliest alteration was loss of striation, mild segmental swelling, and barely visible basophilia. This rapidly

changed to granular necrosis of the sarcoplasm, the appearance of numerous small basophilic bodies, and the accelerated deposition of calcium. Sarcolemmal proliferation around affected fibers appeared at varying times, rarely so pronounced as to obscure the underlying degeneration. A dilatory inflammation was obvious at a still later phase. Neither of the above visceral lesions was seen in animals receiving cortisone alone, or the virus intracerebrally without cortisone.

Discussion

Cortisone has been shown to enhance disease processes in a variety of susceptible hosts. It is noteworthy that in no instance has the hormone made possible a viral infection in a totally refractory species. Cortisone modifies partial refractoriness by shortening the incubation time and increasing morbidity and mortality.[1,3,9-19] It appears, therefore, from the evidence thus far available, that no matter to what degree the disease pattern is expanded or altered by cortisone an inherent ability of the agent to establish infection in the host must exist before the hormone can produce its augmentation. An apparent exception to this generalization exists in the work of one of us[3] who reported poliomyelitis infection of the cortisone-treated hamster by the intraperitoneal route. No clinical infection is evident when cortisone is withheld. In this instance, however, the hamster is partially susceptible when the virus is introduced directly into a tissue amenable to infection, *i.e.*, the central nervous system. Here cortisone may facilitate virus spread from the peritoneal cavity to the spinal cord, a dissemination which would normally be blocked by local defenses. Thus, the apparent contradiction can be resolved by considering the problem of intraperitoneal inoculation as one of viral transportation to susceptible tissue. Assuming an inherent susceptibility, it can be surmised that cortisone allows the utilization of portals of entry which would be otherwise unsuitable. Indeed, careful histologic studies show that very rarely a hamster may develop occult spinal cord lesions after intraperitoneal inoculation of the virus in the absence of cortisone. The experiments of Kilbourne and Horsfall[20] are also of interest in this connection. The authors showed that Coxsackie virus produces a widespread disease in cortisone-treated adult mice similar to that seen in normal suckling mice. It was initially thought that this virus had no effect upon adult mice, but Pappenheimer, Kunz, and Richardson[21] detected pancreatic lesions in adult mice exposed to the virus. Thus, cortisone produces a disseminated disease in a partially refractory adult mouse. It may be seen from studies embodied in this paper that

almost all histologic patterns considered representative of poliomyelitis are found in the cortisone-treated hamster following intracerebral inoculation of the virus. The observations suggest that cortisone does not transform, but exaggerates, experimental poliomyelitis in the manner described in the following paragraphs.

Within the time limits of the experiments (10 days), the perceptible reactors to needle tract injury and viral polio-encephalitis are the leukocytes, arachnoid cells, blood vessels, and microglia, all elements of mesodermal descent. No significant astrocytic changes are evident in the control or experimental groups. Meningitis, commencing about 18 hours after intracerebral inoculation of the virus, is clearly demonstrable when cortisone is withheld. It is only by the sixth day that arachnoidal exudation appears in the cortisone-treated group. This is in spite of the obviously more intensive encephalopathy induced in the latter group. The quiescent appearance of blood vessels adjacent to the traumatic and viral lesions in cortisone-treated hamsters brings out the extensiveness of mesodermal inhibition. Cortisone was previously shown to decrease capillary permeability.[22,23] This may explain in part the paucity of parenchymal edema found near the cerebral needle tracts in animals receiving cortisone. Thus the outstanding feature is the indolence exhibited by mesodermal elements in the presence of obvious neighboring necrobiosis. Under unaltered circumstances, widespread changes accompany neuronal destruction. In contrast, when cortisone is given the initial phase of neuronolysis is unattended by cellular reaction. Observations recorded here demonstrate lympholysis but no change in the number of fixed phagocytic elements. It seems unlikely that products of cellular degeneration induced by the virus are of an essentially different nature in the presence of cortisone. The evidence that the reaction to neurocytolysis takes place belatedly would indicate that the stimulation is present but that the inability to react is the crucial feature of the inhibitory process.

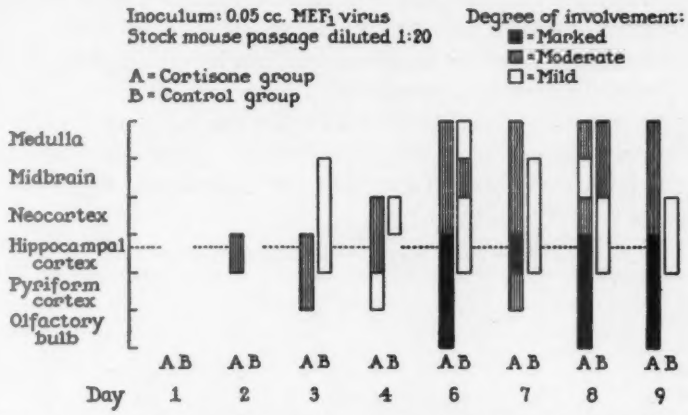
A congeries of responses, collectively grouped under the term inflammation, is considered to be characteristic of the pathology of poliomyelitis; these are neuronophagia, inflammatory infiltration, and perivascular cuffing. The question has been raised as to whether these changes are of a primary or secondary nature, whether the virus directly incites inflammatory response, or whether its essential activity is neuronal necrosis with the inflammation evoked by the products of necrobiosis. Rivers[24] stated that frequently it is impossible, because of the complexity of tissues involved, to ascertain the primary changes induced by the activity of viruses. Howe and Bodian[25] demonstrated,

by injecting the poliomyelitis virus into an area devoid of neurons, that the virus has no effect upon glial cells, but travels inconspicuously to areas rich in neurons where it displays the typical picture of poliomyelitic infection. In discussing this subject Rivers concluded that inflammation is a secondary phenomenon in virus diseases. Studies reported here show that, while cortisone inhibits the components of mesodermal inflammation, the neuronal picture of poliomyelitis is very pronounced. Howe and Bodian found that this virus does not affect glia when neurons are absent. From these two findings it can be surmised that the essential property of poliomyelitis virus is to induce primary neuronal necrosis and that leukocytic, microglial, and endothelial responses are not primary expressions of the action of the pathogen but are merely consequent to tissue destruction. There is ample evidence that cortisone hinders reactive and reparative elements in the face of tissue damage.[26] While published studies do not allude to activity of the central nervous system under the influence of cortisone, it would be inconsistent to assume that the mesodermal structures within the cranial cavity are endowed with distinctive properties which preclude an equivalent response.

Our studies at daily intervals have delineated a progressive pattern of viral lesions under the influence of cortisone. The first observable changes were located in the hippocampal gyrus. From this level there was a steady spread caudally, and in the case of cortisone-treated animals, a rostral dissemination as well (Text-figure 1). On the third day following intracerebral inoculation lesions were restricted to the hippocampus, a site in rodents often affected by encephalitis of diverse causes. These zones of neuronolysis were unattended by cellular reaction. By the eighth day lesions were evident at all encephalic levels, including the olfactory bulbs. Lesions in the latter location, however, evoked a florid cellular reaction. It is evident that the response to neuronal necrosis is inversely proportional to the age of the lesion. Thus, since the hippocampal lesions were the oldest, no response was manifest even in the same animal which demonstrated a characteristic reactive module in a more recent (olfactory bulb) lesion. Since dissemination commenced from the hippocampal cortex (the approximate inoculum depot), it can also be reasoned that the cellular reaction to necrosis is directly proportional to the distance from the portal of entry. On the eighth day, for example, the host defenses were such as to allow response to olfactory lesions, but exudation and neuronophagia were not manifest in the hippocampus at this time. One can conjecture that the stimulus elaborated at the time of hippo-

campal cell lysis (third day) had been effectively dissipated when the potentially responsive cells were capable of activation (eighth day).

Another feature of the intracerebral infection of cortisone-treated hamsters which is not demonstrable in the absence of cortisone is the ability of the virus to extend rostrally from the inoculum depot. Beyond the fifth day, the cortisone animals showed extensive lesions of the primary and secondary olfactory centers. The topographic distribution of the lesions clearly indicated that the virus followed a retro-



Text-fig. 1. Distribution of lesions and intensity of involvement in relationship to elapsed time after inoculation.

grade pathway, commencing in the tertiary rhinencephalic nuclei and ending in the olfactory bulbs. In this latter site the damage was so extensive as to disrupt totally the concentric architecture.

Olfactory bulb involvement is not usually seen in simian poliomyelitis except when the olfactory nerve receptors are exposed to the virus.[25] Under unaltered conditions, poliomyelitis virus has been considered incapable of retrograde ascendancy through the olfactory system. Investigations in this laboratory on cortisone-altered poliomyelitis in monkeys have shown that typical lesions can be established in olfactory bulb tissue regardless of the route of viral entry. This would tend to cast some doubt upon the contention that the portal of entry can be determined by exclusion or inclusion of lesions in certain regions of the central nervous system.

It is noteworthy that there is no ubiquity of central nervous system lesions under the influence of cortisone. From our studies it would seem that only those neural sites potentially susceptible to the virus are affected. Olfactory bulb tissue has no inherent resistance to the

virus. Its usual failure to be implicated when infection is obtained by routes other than intranasal may indicate merely the inability of the virus, in the concentration achieved through intraneural proliferation, to traverse the axonal channels to the olfactory bulb. Since cortisone permits greater intracerebral viral multiplication than in control animals,[1] these transportational difficulties may be surmounted. Some related viruses can affect primary rhinencephalic nuclei after cerebral inoculation. Wolf's[27] detailed description of the anatomical pattern displayed by the Columbia SK virus in rats indicates a fairly constant involvement of these structures.

The finding of lesions in muscle and fat can be explained by several hypotheses. The temporal relationships between lesions of the brain, cord, fat, and muscle (Table IV) lead to the conclusion that a definite viral progression exists. It would appear that segmental myelitis must precede involvement of the corresponding metameres. From this it

TABLE IV
Degree and Extent of Certain Visceral Lesions

Day	1		2		3		4		6		7		8		9		10	
Group	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
Lesion																		
Myositis	0*	0	0	0	0	0	0	0	2	0	2	0	3	0	0	0	–†	0
Steatitis	0	0	0	0	0	0	±	0	2	0	3	0	4	0	1	0	–	0
Myelitis	0	0	0	0	1	0	1	0	3	2	3	0	4	4	2	0	–	0

* Numbers express degree of severity and extensiveness of lesions, 4 being most severe.
† No animals in group A survived 10 days.
Animals in groups A and B were inoculated intracerebrally with 0.05 ml. of MEF_1 diluted 1:20 (mouse pool III).
Animals of group A were given intramuscularly 5 mg. of cortisone simultaneously with the virus.

follows that the observed soft tissue lesions are of a neural egressive nature. The absence of a demonstrable viremia during the limits of the experiment casts some doubts on a presumption of vascular spread, following intracerebral inoculation. Faber, Silverberg, Luz, and Dong[28] have demonstrated the ability of the poliomyelitis virus to emerge from the central nervous system of monkeys and travel by centrifugal spread through axons. The most plausible conjecture regarding the somatic lesions herein described is that they represent still further emergence of the virus into amenable tissue immediately distal to peripheral nerve endings. A strong point in favor of this assumption is the bizarre localization of the lesions within the tissue structures immediately adjacent to the vertebral column. It is evident that those soft tissue zones at the terminus of the shortest emergent motor nerves would be the first to present visible lesions. The absence of changes in

more peripheral musculature might be explained by the rapidly fatal course of the disease which prevents any further spread. The recovery of large concentrations of poliomyelitis virus from identical lesions in hamsters infected by the intraperitoneal route tends to substantiate the contention that the changes in muscle and fat are the direct result of local viral invasion.[29]

The morphologic changes seen in the paravertebral myositis are essentially intracellular, often segmental, and in the initial phases quite isolated. The typical appearance is that of single fibers undergoing granular necrosis, separated by masses of normal fascicles. There is a remarkable similarity of this myopathy to those induced by Coxsackie, MM, encephalomyocarditis, and Theiler viruses. Contamination with these viruses has been eliminated by appropriate animal inoculation and serum neutralization tests.

Summary

Intramuscular administration of cortisone concurrently with intracerebral inoculation of MEF_1 poliomyelitis virus into the Syrian hamster significantly enhances the resultant disease. A comparative morphologic survey sheds some light upon the nature of this augmentation.

A number of histologic differences are apparent. In the control group there is a prompt cellular and vascular reaction to the cerebral needle tract injury. A polymorphonuclear leukocytic response is visible within 18 hours. Endothelial hyperplasia in neighboring vessels is conspicuous by the second or third day. Microglial activation is apparent by the third day. In the cortisone-treated animals, these defensive mechanisms are not apparent during the initial days of infection.

Hamsters receiving no cortisone usually show a topographically limited viral infection, reflected by minimal neuronal damage, limited involvement of contralateral nuclear groups, and, in contrast, an exuberant interstitial inflammatory, perivascular and microglial reaction. Animals treated with cortisone, on the other hand, fail to manifest the usual inflammatory response to infection until the fifth or sixth day, and in subsequent days this reaction is perceptibly subdued. Neuronal destruction, however, is dramatically widespread, usually bilateral and observed at an earlier date than in the control group.

The retarding effect of cortisone upon the defensive responses of the central nervous system appears to be non-specific since similar inhibition is evident when the intracerebral inoculum is a pathogen-free brain emulsion.

Lesions in skeletal musculature and adipose tissue are demonstrable during the later phases of the enhanced disease. These extraneural alterations following intracerebral inoculation of the virus are believed to be the direct result of viral activity.

REFERENCES

1. Shwartzman, G. Enhancing effect of cortisone upon poliomyelitis infection (strain MEF_1) in hamsters and mice. *Proc. Soc. Exper. Biol. & Med.*, 1950, **75**, 835–838.
2. Shwartzman, G., and Fisher, A. Alteration of experimental poliomyelitis infection in the Syrian hamster with the aid of cortisone. *J. Exper. Med.*, 1952, **95**, 347–362.
3. Shwartzman, G. Poliomyelitis infection in cortisone-treated hamsters induced by the intraperitoneal route. *Proc. Soc. Exper. Biol. & Med.*, 1952, **79**, 573–576.
4. Thomas, L., and Mogabgab, W. J. Hemorrhagic skin lesions produced by intradermal meningococcus toxin in rabbits following treatment with ACTH or cortisone. *Proc. Soc. Exper. Biol. & Med.*, 1950, **74**, 829–832.
5. Michael, M., Jr., and Whorton, C. M. Delay of the early inflammatory response by cortisone. *Proc. Soc. Exper. Biol. & Med.*, 1951, **76**, 754–756.
6. Kabat, E. A., Wolf, A., and Bezer, A. E. Effect of cortisone on experimental acute disseminated encephalomyelitis. *Federation Proc.*, 1951, **10**, 412.
7. Mahon, G. S. Stain for myelin sheaths in tissues embedded in paraffin. *Arch. Neurol. & Psychiat.*, 1937, **38**, 103–107.
8. Churg, J., and Grishman, E. Phase microscope studies of renal glomeruli. Glomeruli deposits in "hyaline" substances. *Am. J. Path.*, 1953, **29**, p. 200.
9. Kligman, A. M., Baldridge, G. D., Rebell, G., and Pillsbury, D. M. The effect of cortisone on the pathologic responses of guinea pigs infected cutaneously with fungi, viruses, and bacteria. *J. Lab. & Clin. Med.*, 1951, **37**, 615–620.
10. Kass, E. H., Ingbar, S. H., Lundgren, M. M., and Finland, M. The effect of ACTH and cortisone on pneumococcal and influenza viral infections in the white mouse. *J. Lab. & Clin. Med.*, 1951, **37**, 780–788.
11. Vollmer, E. P., and Hurlbut, H. S. Ineffectiveness of cortisone therapy in mice infected with Japanese B encephalitis and the adverse effect of high doses. *J. Infect. Dis.*, 1951, **89**, 103–106.
12. Southam, C. M., and Babcock, V. I. Effect of cortisone, related hormones, and adrenalectomy on susceptibility of mice to virus infections. *Proc. Soc. Exper. Biol. & Med.*, 1951, **78**, 105–109.
13. Findlay, G. M., and Howard, E. M. The effects of cortisone and adrenocorticotrophic hormone on poliomyelitis and on other virus infections. *J. Pharm. & Pharmacol.*, 1952, **4**, 37–42.
14. Ainslie, J. D., Francis, T., Jr., and Brown, G. C. ACTH in experimental poliomyelitis in monkeys and mice. *J. Lab. & Clin. Med.*, 1951, **38**, 344–358.
15. Antopol, W., and Quittner, H. The changing pattern of infectious processes under the influence of cortisone. *J. Mt. Sinai Hosp.*, 1952, **19**, 91–105.
16. Sabin, A. B. Genetic, hormonal and age factors in natural resistance to certain viruses. *Ann. New York Acad. Sc.*, 1952, **54**, 936–944.
17. Syverton, J. T., Werder, A. A., Friedman, J., Toth, F. J., Jr., Graham, A. B., and Mira, O. J. Cortisone and roentgen radiation in combination as synergistic agents for production of lethal infections. *Proc. Soc. Exper. Biol. & Med.*, 1952, **80**, 123–128.

18. Smith, J. M., Murphy, J. S., and Mirick, G. S. Effect of adrenal hormones on infection of mice with pneumonia virus of mice (PVM). *Proc. Soc. Exper. Biol. & Med.*, 1951, **78**, 505–510.
19. Foster, C., Sigel, M. M., Henle, W., and Stokes, J., Jr. The influence of ACTH on the resistance of monkeys to the Yale SK strain of poliomyelitis virus. *J. Lab. & Clin. Med.*, 1951, **38**, 359–362.
20. Kilbourne, E. D., and Horsfall, F. L., Jr. Lethal infection with Coxsackie virus of adult mice given cortisone. *Proc. Soc. Exper. Biol. & Med.*, 1951, **77**, 135–138.
21. Pappenheimer, A. M., Kunz, L. J., and Richardson, S. Passage of Coxsackie virus (Connecticut-5 strain) in adult mice with production of pancreatic disease. *J. Exper. Med.*, 1951, **94**, 45–64.
22. Ebert, R. H. In vivo observations on the effect of cortisone on experimental tuberculosis, using the rabbit ear chamber technique. *Am. Rev. Tuberc.*, 1952, **65**, 64–74.
23. Cook, C., and MacDonald, R. K. Effect of cortisone on the permeability of the blood-aqueous barrier to fluorescein. *Brit. J. Ophth.*, 1951, **35**, 730–740.
24. Rivers, T. M. Lane Medical Lectures: Viruses and Virus Diseases. Stanford University Press, Stanford University, Calif., 1939, pp. 42–43.
25. Howe, H. A., and Bodian, D. Neural Mechanisms in Poliomyelitis. Commonwealth Fund, New York, 1942, pp. 30–34; 99.
26. Ragan, C., Howes, E. L., Plotz, C. M., Meyer, K., Blunt, J. W., and Lattes, R. The effect of ACTH and cortisone on connective tissue. *Bull. New York Acad. Med.*, 1950, **26**, 251–254.
27. Wolf, A. Studies in rodent poliomyelitis. IV. The pathology of murine and cavian poliomyelitis. *J. Exper. Med.*, 1942, **76**, 53–72.
28. Faber, H. K., Silverberg, R. J., Luz, L. A., and Dong, L. Studies on entry and egress of poliomyelitis infection. III. Excretion of the virus during the presymptomatic period in parenterally inoculated monkeys. *J. Exper. Med.*, 1950, **92**, 571–589.
29. Shwartzman, G., and Aronson, S. M. Poliomyelitis Infection by Parenteral Routes Made Possible by Cortisone. Presented at the Conference on Mechanism of Corticosteroid Action in Disease Processes, October 4, 1952, New York Academy of Sciences.

[*Illustrations follow*]

LEGENDS FOR FIGURES

FIG. 1. Microscopic appearance of needle tract in hamster brain 4 days after inoculation of MEF_1. No cortisone. Cellular response and obliteration of traumatic defect may be noted. Hematoxylin and eosin stain. × 190.

FIG. 2. Microscopic appearance of needle tract in hamster brain 4 days after inoculation of MEF_1. Cortisone (5 mg.) given intramuscularly. The outline of the needle tract was still apparent. Resolution of hemorrhage and cellular reaction are poor. Hematoxylin and eosin stain. × 190.

FIG. 3. Endothelial hyperplasia in small cerebral artery near MEF_1 inoculation site. Hamster sacrificed 3 days after intracerebral injection. No cortisone. Hematoxylin and eosin stain. × 190.

FIG. 4. New capillary formation at site of inoculation 4 days after intracerebral injection of MEF_1. No cortisone. Hematoxylin and eosin stain. × 190.

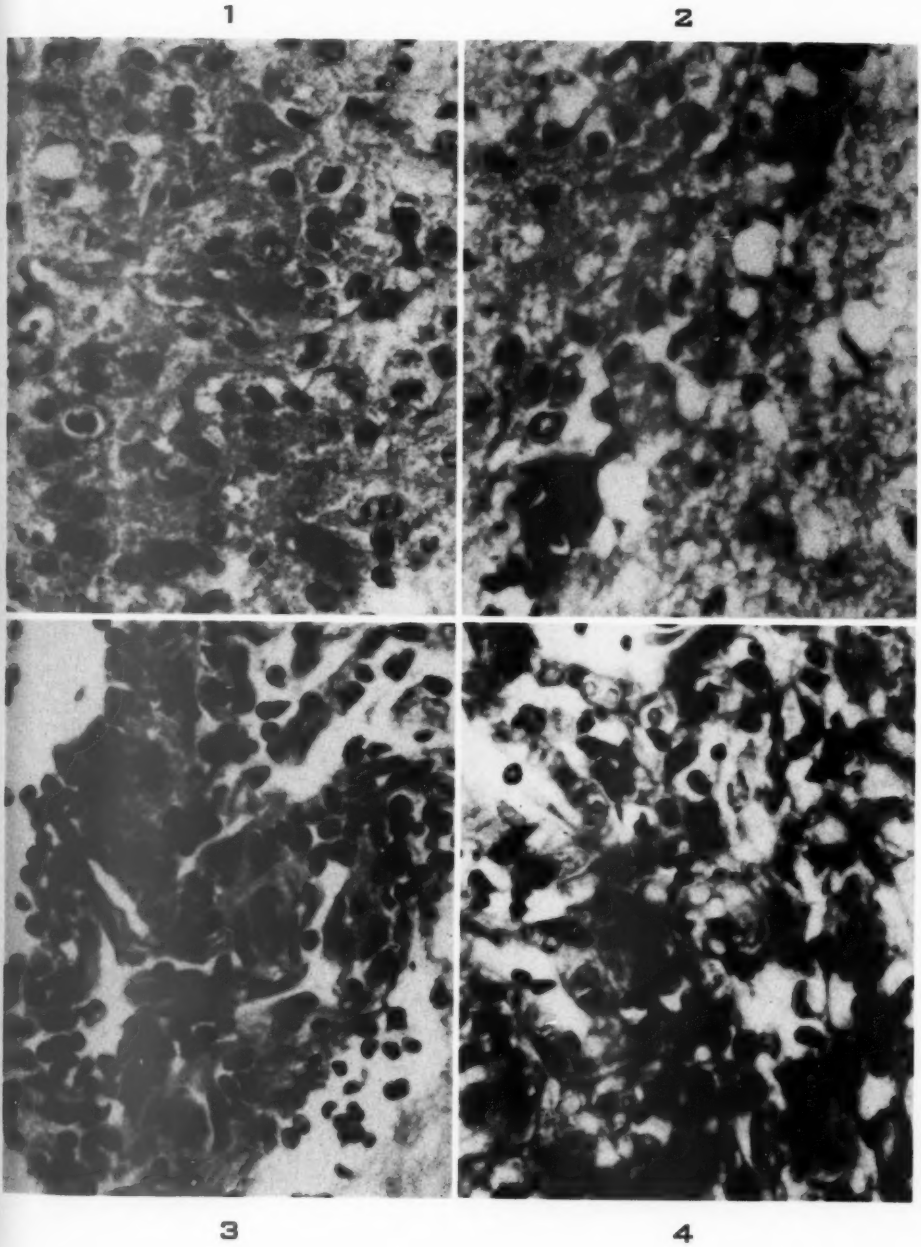

1
2
3
4

FIG. 5. Hippocampal cortex in cortisone-treated hamster sacrificed 7 days after intracerebral inoculation of MEF_1. Diagonal line represents former site of cortical neurons. Negligible inflammatory or vascular response. Hematoxylin and eosin stain. × 70.

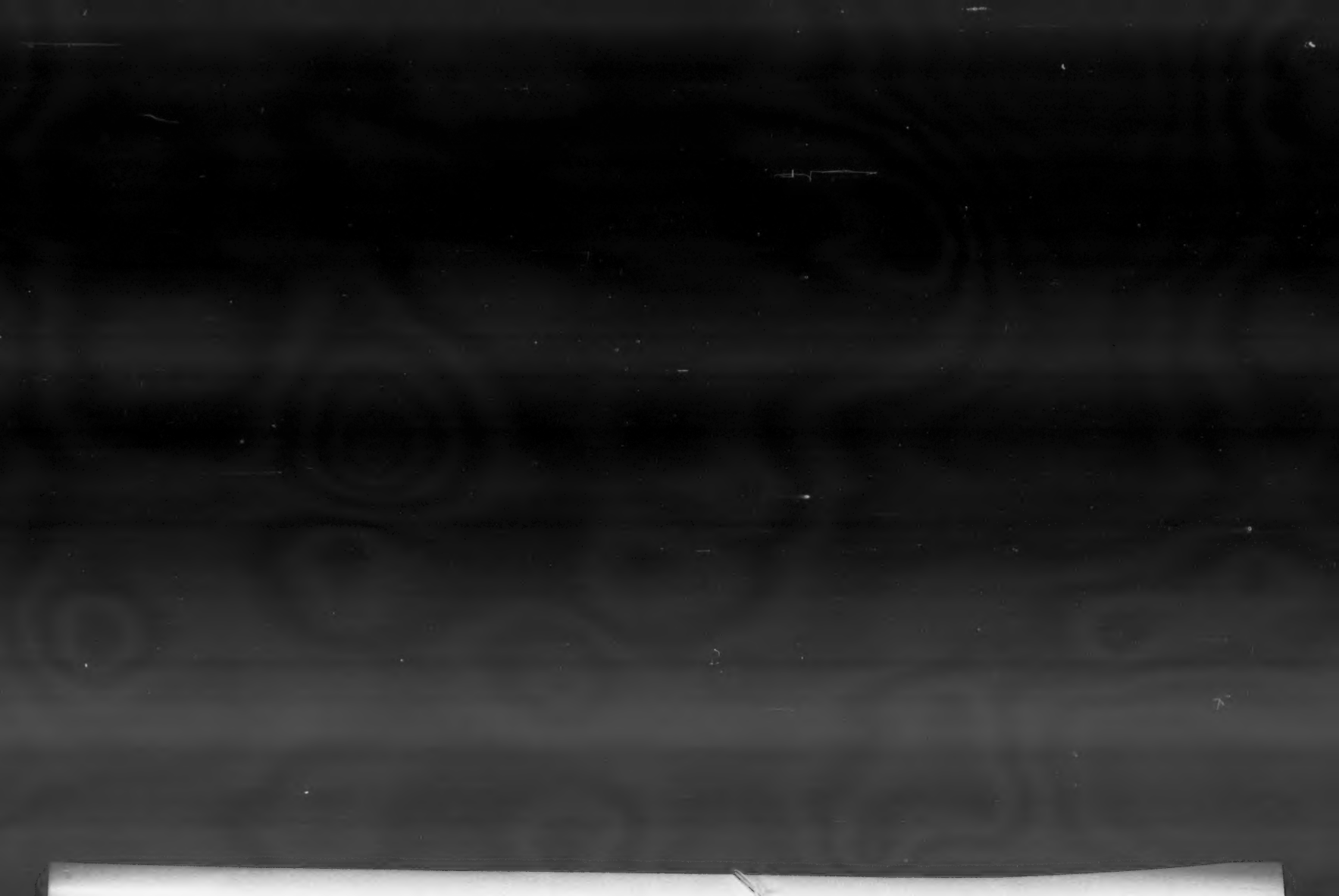
FIG. 6. Hippocampal cortex in hamster sacrificed 7 days after intracerebral inoculation of MEF_1. Perivascular cuffing and inflammatory infiltrates may be noted. The cortical neurons in upper left portion of the field are largely intact. No cortisone. Hematoxylin and eosin stain. × 70.

FIG. 7. Olfactory bulb. Normal microscopic appearance. × 70.

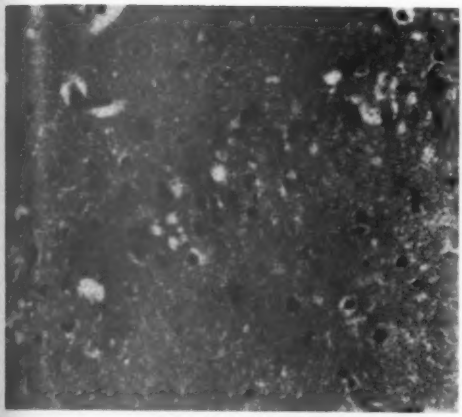
FIG. 8. Olfactory bulb in cortisone-treated hamster sacrificed 9 days after intracerebral inoculation of MEF_1. There is extensive necrosis of parenchyma with uninhibited inflammatory reaction. Hematoxylin and eosin stain. × 70.

FIG. 9. Spinal cord in cortisone-treated hamster sacrificed 8 days after intracerebral inoculation of MEF_1. Total neuronal destruction, but little inflammatory response. Animal tetraplegic. Thionine stain. × 70.

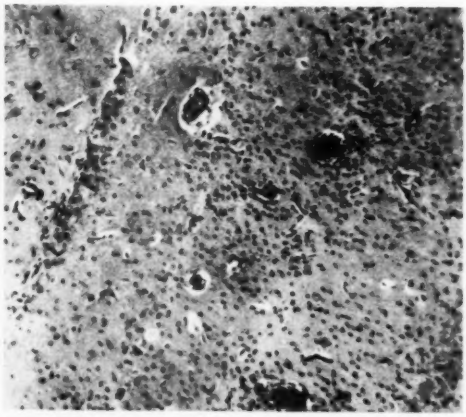
FIG. 10. Spinal cord in hamster sacrificed 8 days after intracerebral inoculation of MEF_1. Moderate neuronal loss. Widespread inflammatory reaction. Clinically, animal showed paralysis of one limb. Thionine stain. × 70.

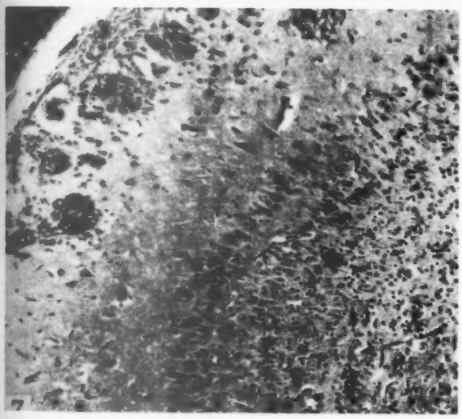


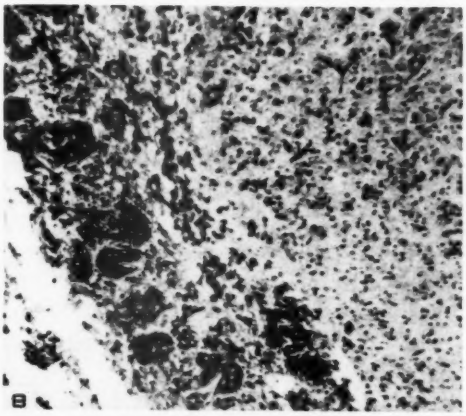
5



6

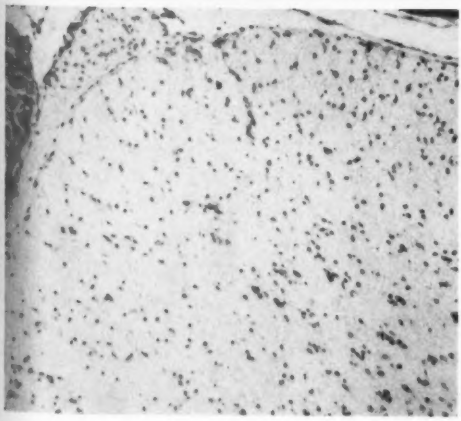




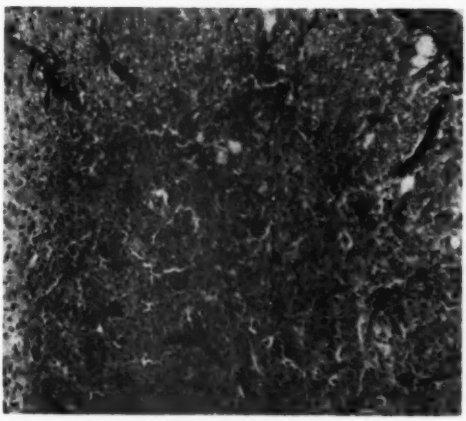


8

8



9



10

THE RELATION OF HERETOFORE UNREPORTED LESIONS TO PATHOGENESIS OF HERPES ZOSTER *

WILLIAM J. CHEATHAM, M.D.

(From the Department of Pathology, Vanderbilt University School of Medicine, Nashville, Tenn.)

The first significant observations relating to the nature and pathogenesis of herpes zoster are generally credited to von Baerensprung,[1] who, by post-mortem observations, established the association of the segmental cutaneous eruption with a lesion of the segmental dorsal root ganglion. This association was substantiated by the extensive observations of Head and Campbell.[2] In two of the cases reported by the latter a peripheral neuritis was described also; others have likewise noted neuritis, though its presence has generally received little attention in the histologic descriptions.[3,4]

That the disease is an infectious one was indicated by the inoculation experiments of Kundratitz[5] and subsequent investigators. Intradermal injection of vesicle fluid results in local vesiculation but does not produce the entire picture of zoster, *i.e.*, segmental neuralgia followed by a belt of vesicles.[6] This discrepancy probably indicates that the skin is not the portal of entry in zoster. The inability to demonstrate bacteria in this infectious vesicle fluid, the nature of the histologic changes in the skin, and the consistent demonstrations of intranuclear inclusion bodies in the cutaneous lesions indicate that the cutaneous lesions are of viral etiology. Some workers, however, have attributed the rash to vasomotor phenomena secondary to the lesion of the dorsal root ganglion.[7] Although it has been reasonable to assume that the lesion of the dorsal root ganglion is due to infection with the virus, there has been no concrete evidence that this is the case.

It is generally assumed by those recognizing the infectious etiology of the disease that the responsible virus travels along the peripheral nerve between the dorsal root ganglion and the skin. This mechanism of spread of virus has been clearly demonstrated in herpes simplex[8] which clinically, histologically, and experimentally has many features in common with herpes zoster. There has not been general agreement as to the direction of travel of the zoster virus along a peripheral nerve, but it appears likely that travel occurs centrifugally from ganglion to skin as supported by Stern.[3] The portal of entry and the means by which the virus gains access to the dorsal root ganglion have not been established.

* Received for publication, November 13, 1952.

The case to be presented gave evidence as to the portal of entry and the manner of spread in the body and established the viral etiology of lesions of the dorsal root ganglion as well as the pathogenesis of certain visceral lesions which may occur with further dissemination of the virus.

Report of Case

M. S. (V.U.H. no. 160,733), a white female, 50 years old, was first seen in Vanderbilt University Hospital in May, 1947, at which time a diagnosis of Hodgkin's disease was made clinically and confirmed by biopsy of an axillary lymph node. During the following 39 months she received repeated x-ray irradiation. Two months following the completion of the last course of irradiation she developed peri-umbilical pain which increased in severity and was more marked on the right. Three days later she was admitted to the hospital, having developed nausea and vomiting following administration of a narcotic.

On admission the skin was clear except for the pigmentation from irradiation. Lymph nodes were not palpable. No abdominal organs were palpable, but there was slight tenderness in the right upper quadrant. Neurologic examination was not remarkable except for a slightly hyperactive patellar reflex on the right.

On the assumption that the pain was due to Hodgkin's disease and since the patient had received all the irradiation that was deemed advisable, she was started on nitrogen mustard therapy on the sixth day of illness. On the eleventh day she developed a band-like, non-pruritic, erythematous, vesiculating rash extending bilaterally from the umbilicus and on the back. At the same time a few isolated vesicles appeared on the extremities. Fluid from abdominal vesicles failed to produce lesions on the chorioallantoic membranes of chick embryos after several attempted passages.[9] On the 15th day she suddenly went into shock and had a clonic seizure of the upper extremities. Immediately thereafter her temperature, which had been normal or slightly subnormal, rose to 104.3° F. The following day she became jaundiced. Abdominal distention developed and the stools gave positive tests for blood. At about this time the abdominal vesicles were noted to be coalescing and vesicles were noted on her face. The latter were not related to the distribution of cutaneous nerves. Some of the vesicles were hemorrhagic. Serum bilirubin and non-protein nitrogen rose and white blood cells fell to 1,050. She remained in shock and expired on the 18th day following the onset of pain.

Necropsy Findings

A prominent vesicular eruption was seen over all parts of the body, with the periphery and base of many of the vesicles hemorrhagic. A broad band of similar but confluent vesicles and bullae coursed from the lower thorax posteriorly on the right about the trunk to the midline where it extended from the level of the lower costal margin to the umbilicus. A band of less confluent lesions was similarly located on the left. The hemorrhagic component of the zoster eruption was less than that of the generalized eruption. The skin was icteric and there was slight generalized pitting edema. Radiation pigmentation was present. All tissues were fixed in Zenker's fluid except the brain which was fixed in formalin. Hematoxylin and eosin stains were used exclusively except as noted.

Microscopic examination of abdominal vesicles showed classical

lesions of herpes zoster (Fig. 1). Type A intranuclear inclusion bodies were readily demonstrable within the epithelial cells in and about the vesicles and were seen rarely within neurilemmal cells of small nerve twigs in the underlying corium. No inclusion bodies were seen within fibroblasts or vascular endothelial cells. There was hemorrhage into the corium underlying the vesicles, but practically no cellular infiltrate was present.

The heart showed only multiple microscopic foci of myocardial necrosis with associated hemorrhages in the myocardium, endocardium, and epicardium.

The lungs were partially atelectatic due to bilateral pleural effusion. Microscopically, small colonies of cocci were seen throughout the sections, with associated local necrosis but without cellular exudation.

The peritoneal cavity contained 1200 cc. of amber-colored fluid, and numerous petechial hemorrhages were seen over the surfaces of the liver and gastro-intestinal tract. There was notable absence of identifiable abdominal lymph nodes, but the retroperitoneal tissues about the lower abdominal aorta, celiac axis, pancreas, and left adrenal gland were firm, yellowish gray, and increased in amount. Retroperitoneal tissues elsewhere were edematous.

The gastric and duodenal mucosa was congested and displayed numerous irregular, superficial ulcers, which in the former location were covered with a shaggy, gray membrane. The wall of the stomach was somewhat scarred. Patches of mucosal hemorrhages were seen throughout the small intestine and in the terminal portion of the colon.

In microscopic sections a small ulcer of the esophageal mucosa was seen adjacent to the esophago-gastric junction. The denuded tissues showed necrosis and polymorphonuclear infiltration and contained unidentified bacillary organisms. No inclusion bodies were seen within the adjacent surface epithelium, but in several of the underlying cardiac glands were foci of necrosis with intranuclear inclusion bodies in both the glandular cells and the squamous epithelium of their ducts (Fig. 2). Throughout the stomach neuritis was evident, involving both the submucosal and myenteric plexuses and manifested by the occurrence of polymorphonuclear leukocytes and nuclear débris along the nerves. At the cardia several of the neurons of the myenteric plexus contained intranuclear inclusion bodies (Fig. 3). Neuritis was not seen elsewhere in the intestinal tract. Changes attributable to irradiation were seen throughout the gastro-intestinal tract. In the mesentery there was hemorrhage along many of the nerves, but no inclusion bodies were identified.

The liver weighed 1840 gm. and in addition to the subcapsular

hemorrhages showed poorly delimited lobules, portions of which were opaque and dull orange. Microscopically these proved to be foci of coagulative necrosis which involved primarily the periportal areas; in some instances entire lobules and groups of lobules were necrotic. All cellular elements were necrotic and no inclusion bodies could be found. The serosa of the gallbladder was edematous and showed mild neuritis; intranuclear inclusion bodies were not seen, but within the cytoplasm of fibroblasts were multiple hyaline, spherical bodies surrounded by clear halos.

The spleen was congested and showed necrosis of the malpighian bodies.

The pancreas was unusually hard and showed fixation of the lobules and a yellowish interlobular infiltrate. Foci of coagulative necrosis were seen throughout both interlobular and intralobular tissues, involving all cellular elements. Many of the acinar cells contained intranuclear inclusions (Fig. 4). Neuritis was not seen.

Within the medulla of one and the cortices of both adrenal glands were foci of necrosis; rare parenchymal cells within the cortical lesions contained intranuclear inclusion bodies and the majority of the cells within the medullary lesions contained these bodies (Fig. 5). At the corticomedullary junction of one adrenal gland was a small focus in which were myriads of intracytoplasmic yeast-like bodies. These organisms showed eccentric crescents of chromatin, stood out prominently with periodic acid stain, and were morphologically identical with *Histoplasma capsulatum*.

The kidneys and urinary bladder showed no pertinent lesions.

The only significant lesion seen within the genitalia was a microscopic focus of necrosis within one of the ovaries. The involved and surrounding cells contained intranuclear inclusion bodies (Fig. 6).

The bone marrow was markedly hypoplastic.

The spinal nerve which appeared to supply the area of zoster eruption on the right was traced to the spinal cord and the latter removed together with both dorsal root ganglia and one of the sympathetic ganglia at this level. No gross or microscopic lesions of the brain or spinal cord were noted. One of the dorsal root ganglia showed massive hemorrhage and necrosis of a large portion of the ganglion cells. Large intranuclear inclusion bodies were seen rarely in ganglion cells; these inclusions were irregular and were larger and more basophilic than those elsewhere (Fig. 7). Acidophilic inclusion bodies were seen within the nuclei of numerous satellite cells. There was no cellular infiltrate. Several small accumulations of cocci were associated with

localized necrosis. The changes in the opposite dorsal root ganglion were less severe and inclusion bodies could be found only in satellite cells. The sympathetic ganglion showed hemorrhage and necrosis which was even more marked than that in the dorsal root ganglia, and intranuclear inclusion bodies were occasionally identified within ganglion cells (Fig. 8).

Although the diagnosis of Hodgkin's disease had been well established prior to death, it was impossible to confirm the diagnosis from necropsy findings. This was undoubtedly due largely to nitrogen mustard therapy. The hypoplasia of the marrow and paucity of cellular infiltration in reaction to foci of necrosis were also attributed to nitrogen mustard.

Discussion

Relation Between Zoster and Varicella

That zoster is due to a virus has now been fairly well accepted. Unfortunately no consistently reproducible procedures for growing the virus have been developed, and it has been impossible to induce the disease in experimental animals. Studies of the virus and its identification have therefore been impossible. It was noted early that cases of zoster could give rise to varicella in contacts and rarely zoster could be contracted from varicella patients.[10,11] Vesicle fluids from the two diseases give rise to identical lesions grossly and microscopically when inoculated into the skin of human volunteers.[12] Other observations also support the identity of the two viruses. The objections to the theory of identity of the causative agent of the two diseases can be resolved if two propositions be accepted: (1) Varicella is a hematogenous manifestation in a non-immune individual while zoster is due to neurogenous spread in a person with humoral immunity, and (2) variations in strains in relation to pathogenicity and cell affinity occur as in other infectious agents.

Pathology of Varicella

Reports of necropsies on patients with varicella are rare. Those available indicate that the disease is a generalized one affecting tissues other than the skin. Focal necrosis is seen within numerous organs, particularly the liver, lungs, spleen, and adrenal glands.[13-17] It is particularly noteworthy that typical inclusion bodies are seen in the endothelium of blood vessels in the lesions. This finding is in keeping with the theory of hematogenous spread of the virus. The disseminated visceral lesions in the case of zoster herewith reported resemble those described in varicella except that no inclusion bodies were seen within

the endothelial cells. Such varicelliform lesions might occur in the course of a zoster infection when generalized immunity has been lost.

Portal of Entry in Zoster

During the prodromal phase of zoster various respiratory and digestive disturbances may occur.[18] Such symptoms are probably indicative of the portal of entry in many instances. In this case nausea and vomiting were present early, but because of the apparent relation to drugs administered, little significance can be attached to them. There was involvement of the mucosa of the esophagus and of the nerves of the esophagus and stomach as evidenced by the presence of inclusion bodies. From the presence of this neuritis it is evident that these lesions were not merely a part of the hematogenous spread of the virus, which apparently accounted for the varicelliform eruption and most of the visceral lesions, but more likely were a part of the neurogenic spread.

Route of Infection of the Dorsal Root Ganglion

One of the problems in zoster has always been how a particular dorsal root ganglion is involved. At times the selection of the ganglion is apparently on the basis of injury to the ganglion or its nerve, with activation of a latent virus. In most instances, however, the location of the visceral portal of entry and the autonomic nerves supplying it probably determine the ganglion involved. The findings in this case fit well the concept that the virus entered the esophagus, passed to the sympathetic ganglion by the visceral nerves, and entered the dorsal root ganglion by the white ramus; in each of these locations (except the white ramus, which was not examined) inclusion bodies were found. It is very unlikely that these lesions were not links of a chain along which the virus spread. Head and Campbell[2] pointed out that the ganglia most frequently affected were those receiving white rami. This theory of spread does not exclude the possibility of infection of nerves other than those receiving white rami, which could become infected by more devious paths, nor infection of cranial nerves by homologous paths.

One bit of evidence indicating that the lesion in the intestinal tract was older than that in other links of this chain of infection is that only in association with the neuritis in the esophagus and stomach was there any cellular infiltrate. The patient must have received nitrogen mustard after the occurrence of the lesion at the portal of entry but before the outbreak of the rash. By the time the lesion in the cutaneous nerves had developed, she had lost much of her ability to react

by out-pouring leukocytes. In other cases it has been noted that the rash appears first at the point at which the cutaneous nerves come to the surface, and spreads therefrom.[3] This would indicate that the spread is centrifugally along the nerve.

Inclusion bodies are described in the endothelial cells of vessels of the skin lesions of zoster as well as of varicella.[6] Virus must be liberated into the blood with death of these cells, and if there were not a humoral immunity in zoster it would be expected that the lesions would generally be widely disseminated.

Kaposi's Varicelliform Eruption

One disease from which this case must be differentiated is Kaposi's varicelliform eruption. From the evidence now available it appears that this disease is, at least in most cases, actually a disseminated herpes simplex. The inability to produce lesions on the very susceptible chorioallantoic membranes of the chick embryo would indicate that the responsible agent in the case described was not the virus of herpes simplex. The clinical history and findings in the dorsal root ganglia are further evidence in establishing the identity of the disease as zoster.

Summary

A case of herpes zoster with varicelliform eruption is presented in which intranuclear inclusion bodies were demonstrated within the esophageal mucosa, myenteric plexus of the stomach, dorsal root ganglia, and a sympathetic ganglion at the level of the affected dorsal root ganglia. In addition, similar inclusions were seen within cells in lesions of the pancreas, adrenal glands, and one ovary, associated with focal necrosis. Such lesions have not been previously described in zoster.

It is postulated that the virus entered the body via the sympathetic nerves of the esophagus and migrated by the sympathetic nerves to the dorsal root ganglia, eventually reaching the skin by centrifugal spread along the respective peripheral nerves. The disseminated visceral and cutaneous lesions resemble those described in varicella.

The viruses of herpes zoster and varicella are probably identical. In varicella the lesions probably result from a hematogenous spread in a non-immune individual, while in typical zoster they are tentatively considered to result from neurogenous spread in a person with humoral immunity.

The respiratory and gastro-intestinal tracts probably serve as portals of entry in most cases of herpes zoster.

An incidental finding in this case was the apparently inconsequential growth of *Histoplasma capsulatum* in one adrenal gland.

REFERENCES

1. von Baerensprung. Beiträge zur Kenntniss des Zoster. (Dritte Folge.) *Charité-Ann.*, 1863, **11**, Pt. 2, 96–116. (Cited by Head and Campbell.[2])
2. Head, H., and Campbell, A. W. The pathology of herpes zoster and its bearing on sensory localisation. *Brain*, 1900, **23**, 353–523.
3. Stern, E. S. The mechanism of herpes zoster and its relation to chicken-pox. *Brit. J. Dermat.*, 1937, **49**, 263–271.
4. Lipschütz, B. Cited by Bruusgaard.[6]
5. Kundratitz, K. Experimentelle Übertragungen von Herpes zoster auf Menschen und die Beziehungen von Herpes zoster zu Varicellen. *Ztschr. f. Kinderh.*, 1925, **39**, 379–387. (See also van Rooyen and Rhodes.[18])
6. Bruusgaard, E. The mutual relation between zoster and varicella. *Brit. J. Dermat.*, 1932, **44**, 1–24.
7. Baird, P. C., Jr. Herpes zoster. *New England J. Med.*, 1943, **228**, 568–577.
8. Goodpasture, E. W., and Teague, O. Transmission of the virus of herpes febrilis along nerves in experimentally infected rabbits. *J. M. Research*, 1923–24, **44**, 139–184.
9. Randall, C. C. Personal communication.
10. v. Bókay, J. Ueber den ätiologischen Zusamnenhang der Varicellen mit gewissen Fällen von Herpes zoster. *Wien. klin. Wchnschr.*, 1909, **22**, 1323–1326.
11. Stokes, J., Jr. Varicella-Herpes Zoster Group. In: Rivers, T. M. (ed.) Viral and Rickettsial Infections of Man. J. B. Lippincott Co., Philadelphia, 1948, pp. 395–400.
12. McCarthy, L. Histopathology of Skin Diseases. C. V. Mosby Co., St. Louis, 1931, p. 133.
13. Johnson, H. V. Visceral lesions associated with varicella. *Arch. Path.*, 1940, **30**, 292–307.
14. Oppenheimer, E. H. Congenital chickenpox with disseminated visceral lesions. *Bull. Johns Hopkins Hosp.*, 1944, **74**, 240–250.
15. Lucchesi, P. F., La Boccetta, A. C., and Peale, A. R. Varicella neonatorum. *Am. J. Dis. Child.*, 1947, **73**, 44–54.
16. Claudy, W. D. Pneumonia associated with varicella; review of the literature and report of a fatal case with autopsy. *Arch. Int. Med.*, 1947, **80**, 185–192.
17. Eisenbud, M. Chickenpox with visceral involvement. *Am. J. Med.*, 1952, **12**, 740–746.
18. van Rooyen, C. E., and Rhodes, A. J. Virus Diseases of Man. Thomas Nelson & Sons, New York, 1948, ed. 2, pp. 155–168.

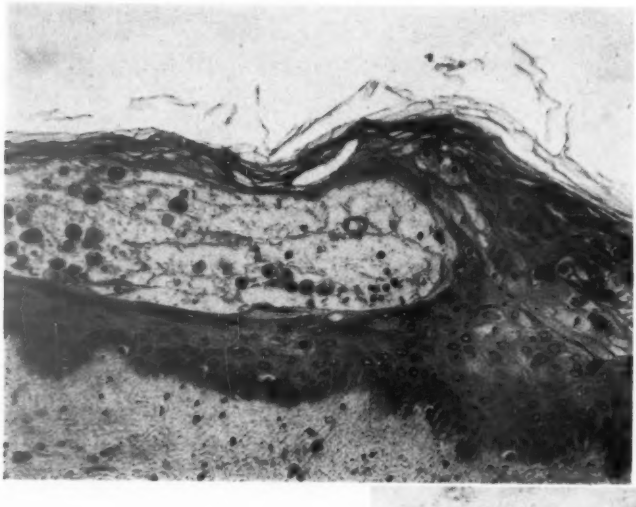
LEGENDS FOR FIGURES

FIG. 1. Intra-epithelial vesicle from zoster eruption of abdominal wall. × 150.

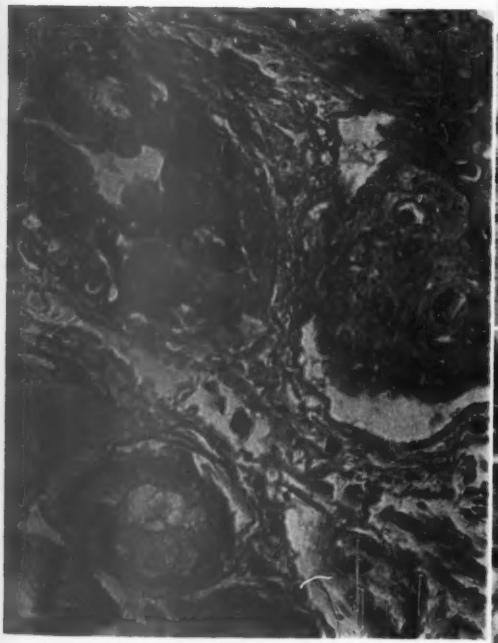
FIG. 2. Esophageal glands bordering gastro-esophageal junction. Intranuclear inclusion bodies are seen within the ducts of these glands. × 450.

FIG. 3. Ganglion cells of myenteric plexus from cardiac end of stomach showing intranuclear inclusion bodies. × 450.

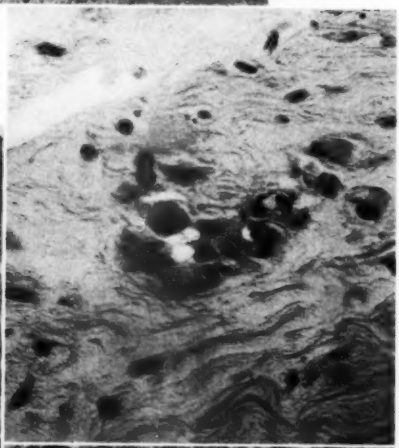
FIG. 4. Pancreas. Necrosis and intranuclear inclusion body within acinar cell. × 300.



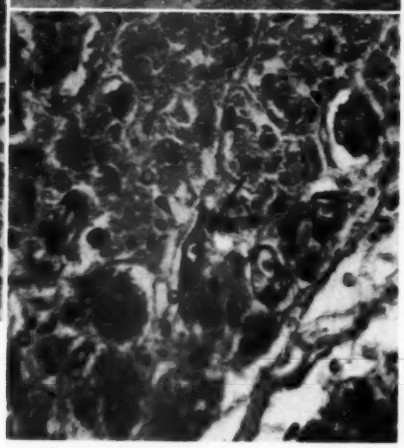
1



2



3



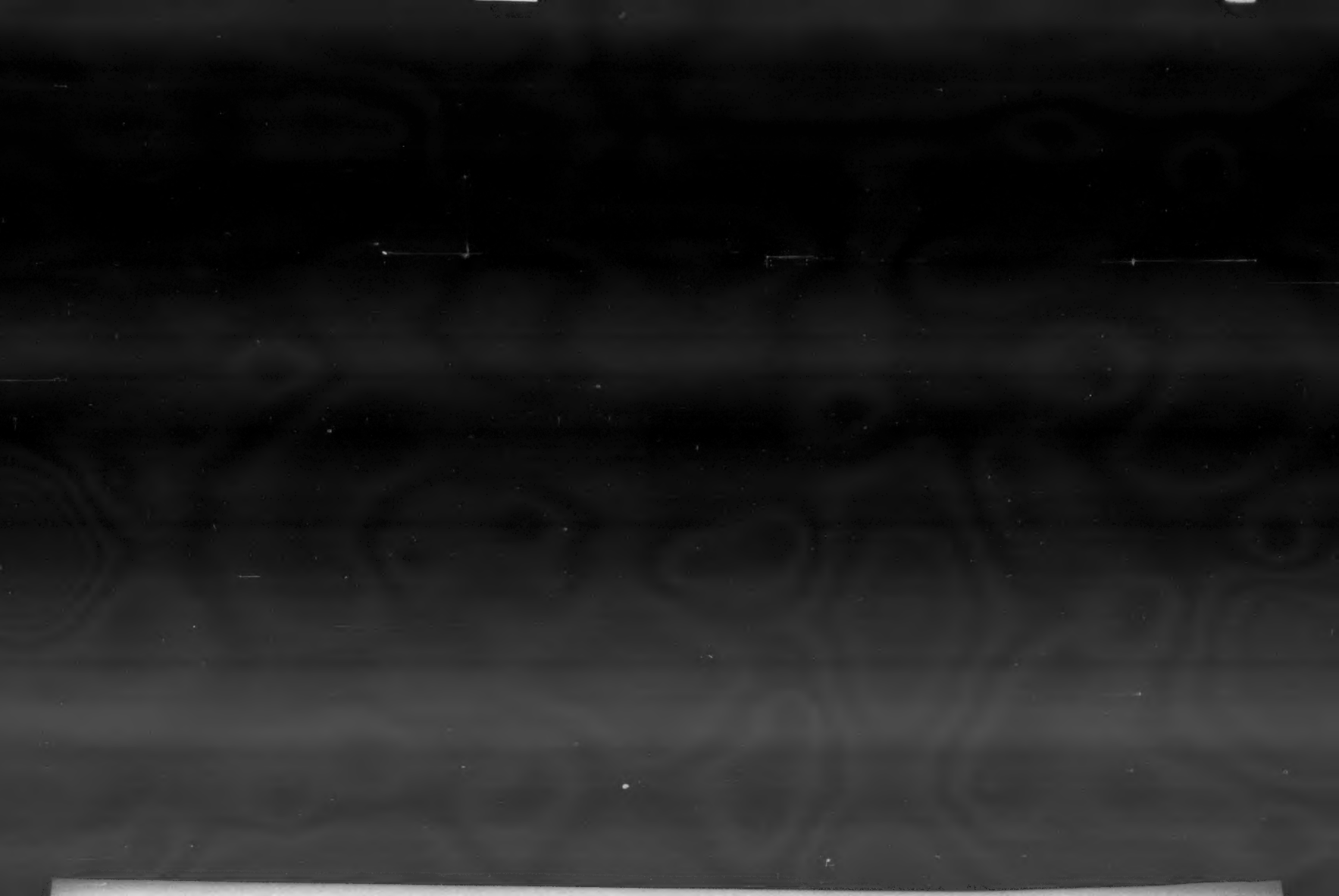
4

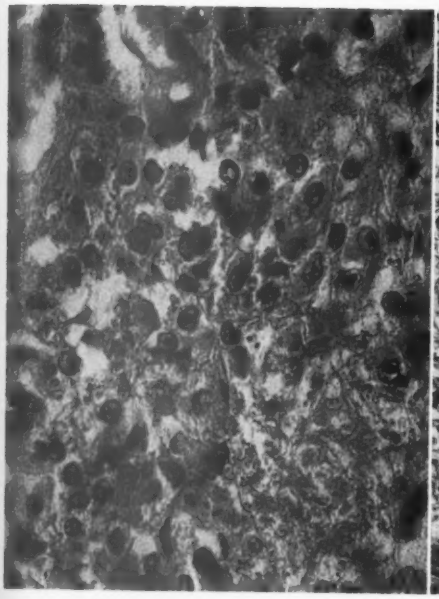
FIG. 5. Adrenal medulla showing several intranuclear inclusion bodies. × 450.

FIG. 6. Ovary with focus of necrosis and intranuclear inclusion bodies. × 675.

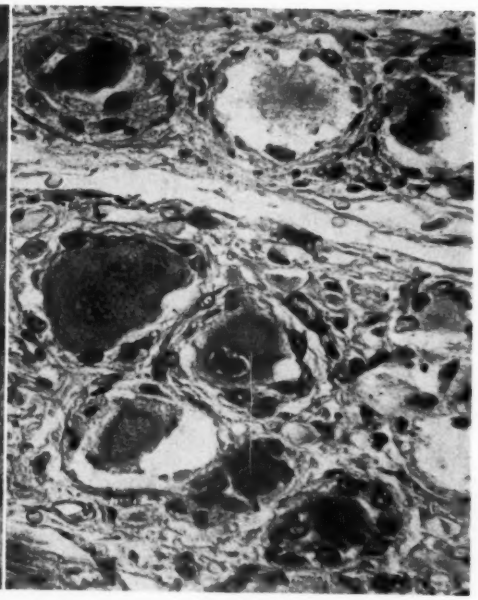
FIG. 7. Dorsal root ganglion with intranuclear inclusion body within ganglion cell. × 300.

FIG. 8. Sympathetic ganglion showing necrosis and hemorrhage, and an intranuclear inclusion body within ganglion cell. × 300.

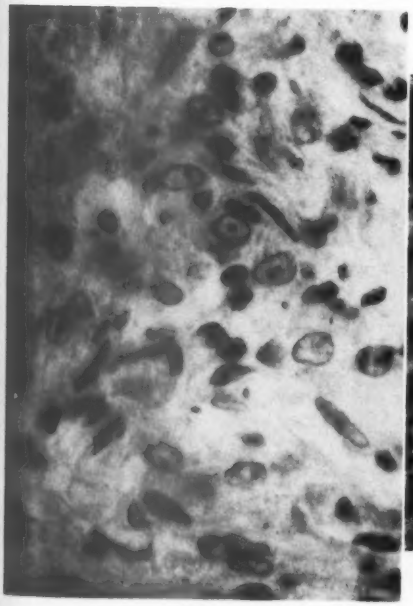




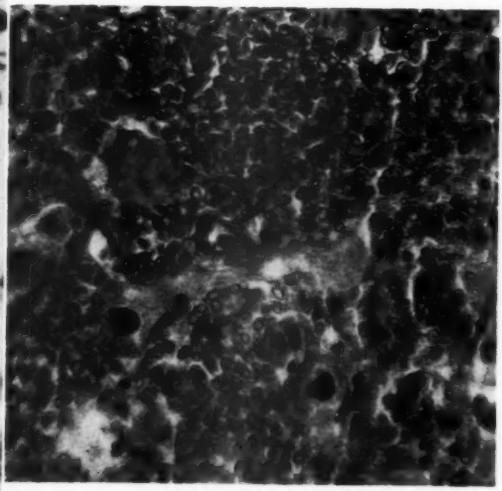
5



7



6



8

PANMYELOSIS AND CHRONIC GRANULOCYTIC LEUKEMIA *

BERNARD BLACK-SCHAFFER, M.D.,† and LELAND D. STODDARD, M.D.‡
(From the Department of Pathology, Duke University School of Medicine, Durham, N.C.)

Myelogenous leukemia is commonly divided, for clinical and pathologic purposes, into groups: acute (myeloblastic) and chronic (myelocytic) leukemia. Among these are included the less common examples of eosinophilic, basophilic, and megakaryocytic leukemias. The differentiation commonly rests upon the identity and relative quantities of leukocytes of various types in the peripheral blood and bone marrow. Only in relatively few institutions are splenic aspiration and needle biopsy of the liver used.

When Arinkin,[1] in 1929, first described his simple method of aspiration of sternal marrow a wave of renewed interest and investigation in hematology was set into motion, second only to that following Neumann's[2] discovery of nucleated red cells in bone marrow (1868) and Ehrlich's adaptation of aniline dyes to hematology (1877). The recent discovery of the action of certain drugs[3-6] upon hematopoiesis is giving rise to a new era of investigation. This, while not primarily morphologic, is nevertheless dependent upon the recognition of changes in the constitution of the marrow, peripheral blood, and viscera. Such investigation, to be effective, requires that the pathologic anatomy of the leukemias be understood and, so far as possible, organized. As to chronic myelogenous leukemia, this understanding and organization seem to be lacking.

MATERIALS AND METHODS

All cases in the files of the Department of Pathology of the Duke University Medical School from 1931 to 1950 labeled as "myelogenous leukemia" were re-examined. These, with remarkably few exceptions, had been diagnosed clinically and pathologically as examples of either "acute" or "chronic myelogenous leukemia." It soon became apparent that "chronic myelogenous leukemia" constituted a heterogeneous group which was subdivided on the basis of pathologic anatomy alone into the categories listed in Table I: chronic granulocytic leukemia (8 cases); panmyelosis without myelofibrosis (3 cases), and with myelofibrosis (4 cases, 2 of which showed only minimal fibrosis); myelofibrotic panmyelosis associated with granulocytic leukemia (3 cases), and with reticuloendotheliosis (1 case); a total of 19 cases.

* Received for publication, October 20, 1952.
† Now at Cincinnati General Hospital, Cincinnati 29, Ohio.
‡ Now at University of Kansas School of Medicine, Kansas City 12, Kans.

Tables II, III, and IV present the pathologic, and some hematologic and clinical data concerning these cases. Three instances of acute granulocytic leukemia originally considered by the clinicians as examples of "chronic myelogenous leukemia" are included for purposes of comparison. An analysis of all instances of acute granulocytic leukemia is not a part of this report.

TABLE I

Tabulated Pathologic Data for the Cases Studied

Categories	Necropsy no.	Bone marrow						Spleen					
		Hyperplasia	Fibrosis	Osteosclerosis	Megakaryocytes	Erythrogenesis	Granulocytes	Weight	Infarcts	Follicles	Megakaryocytes	Erythrogenesis	Granulocytes
								gm.					
	285	+++	0	0	±	++	+++		0	+	±	±	+++
	1816	+++	0	0	±	±	+++*	1050	0	++	±	0	+++*
Chronic granulocytic leukemia	3110	+++	0	0	±	±	+++	660	0	±	0	±	+++
	3538	+++	0	0	±	+	+++*	450	0	++			
	3677	+++	0	0	+	++	+++	780	0	+	0	±	+++
	5288	+++	0	0	±	++	+++	2530	+++	0	±	+	+++
	5380	+++	0	0	±	±	+++	2700	0	±	±	+	+++
	5818	+++	0	0	±	±	+++	395	0	++	0	0	++
Panmyelosis without myelofibrosis	474	+++	0	0	+++	++	++	4000	0	0	+++	++	++
	2231	+++	0	0	+++	+++	++	820		+	±	0	++
	2848	+++	0	0	+++	++	+++	2335	0	0	++	±	+++
Panmyelosis with myelofibrosis	3125	+++	±	0	+++	++	++	1400	0	0	+++	++	++
	3555	+++	±	0	++	+++	++	700	0	+	+++	+++	++
	4964	±	+	++	+++	++	++	4200	+	0	++	++	+++
	5335	0	+++	+++	0	0	+	2850	+	+	++	+++	+++
Transition cases	2576	+++	+	0	+++	+++	+++	4500	++	+	+++	+++	++
	3360	++	++	+	±	±	++†	3000	+++	+	++	++	++†
	4326	+++	+++	+	+	±	+++†	2600	++	0	±	0	+++†
	5861	++	++	++	+++	+++	++‡	2530	+	0	+++	+++	+++‡
Myeloblastic leukemia	3081	+++	0	0	±	0	+++	1550	0	+	+	0	++
	3745	+++	0	0	+	0	+++	570		+	0	0	+++
	4663	+++	0	0	0	0	+++	910	0	+	0	0	+++

* Numerous eosinophils, including eosinophilic giant cells, some of which are multinucleated.

† Atypical primitive cells (hematologically myeloblasts).

‡ Myeloblasts in focal areas.

§ The values (+, +++, +, ++) apply to cervical lymph nodes. The values for mesenteric lymph nodes were: +++, +, O, ++, respectively.

For some cases previously diagnosed as "chronic myelogenous leukemia," the term *panmyelosis* was adopted in lieu of the multiple names extant, because it is a simple statement of the essential features that will be described: a generalized proliferation of marrow tissue—granulocytic, erythrocytic, megakaryocytic, and sometimes even connective tissue—both within and without the marrow cavities. The term avoids focusing attention upon individual organs or upon the peripheral hemogram, which may be very similar to, if not at times identical with, that of chronic granulocytic leukemia. The term is

short. The stem, myelosis, indicates a relationship to other similar diseases. In this paper, the term *myelosis*, rather than "myelogenous leukemia," is used as the generic term referring to the entire group of diseases under discussion. In fact, it would be rational to apply the designation myelosis with appropriate modifiers to all the related diseases. This would eliminate the ambiguity of the term "leukemia,"

TABLE I (Continued)

	Liver					Lymph nodes				Involvement of other regions						
Weight	Portal fields	Sinusoids	Megakaryocytes	Erythrogenesis	Granulocytes	Involvement	Megakaryocytes	Erythrogenesis	Granulocytes	Kidney	Renal pelvis	Heart	Adrenal gland	Pancreas	Testis	Skin
gm.																
2100	++	+++	+	±	+++	++	0	0	+++	+++		++			+++	
3000	+++	+++	±	0	+++*	++	±	±	+++*	±			±			
3100	+++	++	0	0	+++	++	±	0	+++		+	+		+		
2440	+++	++	0	0	+++*	++	±	0	+++*	++			+			
3000	±	±	±	0	±	+	0	0	++	+		0	0		0	0
2300	±	±	±	0	++	++	+	++	++	0		0	0	0		0
2820	+++	++	0	0	+++	++	0	0	+++	+		+	+			
2150	+	++	0	0	++	++	0	0	++	+			±	+		
	+	+++	++	++	++	+	+++	+	++	0	±	0	±	0	0	0
2100	0	+	+++	+	+	+	++	0	+	0		0	0	0		
2735	+	++	+++	0	+++					0		0	0			
2900	±	+	+++	±	++	+	++	0	++	0		0		0		
1800	0	±	+++	0	0	+	+++	+	++	0		0	0	0	0	0
7000	0	+++	+++	+++	±	++	+++	++	+++	0			0	0		
6000	±	+++	+++	+++	+++	+	++	0	++	0	+		±	±		
2400	0	+	+++	++	+	+§	+++§	+§	++§	0		0	0	0		
3290	++	++	+++	+++	++	++	++	++	++†	+		+		0		
2680	0	++	+	±	+++†	++	++	0	+++†	±		0		0		
2530	±	+	±	±	++‡	+++	+	+	+++‡	+++	+++	+	+	+		
2510	+++	+	+	0	+++	+++	±	0	++	+++						
2200	+++	+	0	0	+++	++	±	0	+++	++		+			+	++
2800	+++	++	0	0	+++	+++	0	0	+++	+		+	+		+	

which could then be reserved simply to describe the state of the peripheral blood. However, in an effort to retain familiar terminology as far as possible we have not done this. In the description of our material that follows, we shall refer to *chronic granulocytic leukemia* and to *acute granulocytic leukemia* rather than to "chronic granulocytic myelosis" and "acute granulocytic myelosis."

THE PATHOLOGIC-ANATOMICAL CHARACTERISTICS OF CHRONIC GRANULOCYTIC LEUKEMIA, ACUTE GRANULOCYTIC LEUKEMIA, AND PANMYELOSIS

Chronic Granulocytic Leukemia

The 8 cases of chronic granulocytic leukemia were characterized by universal *bone marrow* hyperplasia, marked by the overwhelming predominance of the myelocyte (Fig. 1). In so far as tissue sections per-

TABLE II

Pathologic-Anatomical Characteristics of Granulocytic Myeloses and Panmyelosis

Types	Acute granulocytic leukemia	Chronic granulocytic leukemia	Panmyelosis without fibrosis	Panmyelosis with fibrosis
Identifying cells	Myeloblast	Myelocyte	All normal marrow elements with prominence of megakaryocytes	
Bone marrow	Homogeneous hyperplasia of myeloblasts with absence of other elements	Hyperplasia of granulocytes with reduced to absent erythropoiesis and megakaryopoiesis	Pleomorphic hyperplasia of all normal elements; megakaryocytosis	Myelofibrosis inversely proportional to pleomorphic hyperplasia of all elements; megakaryocytosis
Spleen	Moderate splenomegaly; replacement by homogeneous proliferation of myeloblasts	Marked splenomegaly; replacement by proliferation of granulocytes	Massive splenomegaly	
			Replacement by pleomorphic proliferation of all marrow elements; megakaryocytosis	plus fibrosis
Liver	Little to moderate hepatomegaly; greater involvement of portal fields than sinusoids		Absent to severe hepatomegaly; greater involvement of sinusoids than portal fields	
	Proliferation of myeloblasts	Proliferation of granulocytes	Pleomorphic proliferation; megakaryocytosis	
Lymph nodes	Pronounced homogeneous replacement by myeloblasts	Little to moderate proliferation of granulocytes; absent to little erythropoiesis; occasional megakaryocyte	Little to moderate pleomorphic proliferation of all marrow cells; megakaryocytosis	plus fibrosis
Other organs	Usually moderate to advanced involvement		Usually absent to minimal involvement	

TABLE III

Hematologic Data

Category	Necropsy number	Peripheral white blood cells	Peripheral Hgb. or red blood cells	Circulating immature red blood cells	Platelets	Hemorrhagic diathesis	Causal factor for hemorrhage	Bone marrow white blood cells
Chronic granulocytic leukemia	285	138,000	7.0 gm.	0	Reduced	0		
	1816	8,200	9.8 gm.	0	25,000	3+	Spontaneous	91,000
	3110	250,000	11.5 gm.	0	15,000	4+	Spontaneous	440,000
	3538	30,800	3.9 gm.	0	0	2+	Spontaneous	
	3677	5,900	8.6 gm.	+	100,000	2+	Spontaneous	200,000
	5288	125,000	8.0 gm.	+	320,000	4+	X-ray	
	5380	352,000	7.8 gm.	0	1,300	0		
	5818	60,000	8.6 gm.	+	48,000	2+	Spontaneous	>1,000,000
Panmyelosis without myelofibrosis	474	195,000	3,000,000	+		0		
	2231	256,000	7.2 gm.	+		+	Tooth extracted	
	2848	77,000	9.4 gm.	+	Normal	0		90,000
Panmyelosis with myelofibrosis	3125	45,000	11.0 gm.	+	Adequate	0		
	3555	187,000	8.8 gm.		Adequate	+	X-ray	216,000
	4964	300,000	5.4 gm.	+	Adequate	0		
	5335	45,000	11.0 gm.		Adequate	0		38,000
Transition cases	2576	125,000	11.3 gm.	0	Adequate	0		
	3360	40,000	10.0 gm.	0	Adequate	0		48,000
	4326	200,000	9.6 gm.	+	Adequate	3+	X-ray	170,000
	5861	15,700	5.9 gm.	+	700,000	0		164,000
Acute granulocytic leukemia	3081	150,000	6.0 gm.	0	40,000	4+	Spontaneous	200,000
	3745	7,000	8.0 gm.	+	100,000	2+	Spontaneous	100,000
	4663	25,500	6.0 gm.	+	50,000	1+	Spontaneous	104,000

mitted study, abnormalities of cellular structure such as may be encountered in the myeloblasts of the acute leukemias were not found. These myelocytes did not appear to be neoplastic cells. Myeloblasts were plentiful, and polymorphonuclear neutrophilic and eosinophilic leukocytes invariably were present. Eosinophilic granulocytes were sometimes so numerous as to suggest eosinophilic leukemia.

Erythropoiesis varied in degree from case to case. It was present in all, but never very prominently, and was often strikingly reduced. Megakaryocytes, although demonstrable, were clearly reduced in number. The reduction appeared to be absolute, and reference to Table III shows a correlated spontaneous reduction of circulating platelets with thrombocytopenic purpura in 5 instances.

The bone trabeculae were thinned. The means by which this was produced was not evident.

The *spleens* of the 8 cases were only moderately enlarged, the average weight of 7 being 1,223 gm. There was a homogeneous replacement of the parenchyma by granulocytic cells like those in the bone marrow (Fig. 2). Megakaryocytes were absent in 3 and rare in 4; erythrogenic foci were scarce. The follicles were markedly reduced in size and number but still recognizable.

The *livers* were of special significance; all were increased in size, averaging 2,613 gm. The most striking histologic change was the presence in the portal fields of the leukemic cells, again chiefly myelocytes (Fig. 3). The sinusoids were likewise the site of leukemic proliferation, but in 3 of the 8 instances they showed distinctly less involvement than the portal fields; in only 2 was the reverse true and in the remainder involvement was about equal. Here, as in the marrow and spleen, megakaryocytes were rare. In 4 cases they were absent. Erythropoiesis was not found in 7 and was slight in one case.

The *lymph nodes* showed moderate involvement in 7 cases and in one case less appreciable replacement by the typical granulocytic proliferation already described (Fig. 4). Erythropoiesis was recognized in only 2 instances, and megakaryocytes were either absent or rarely seen.

Involvement of viscera other than those already discussed characterizes chronic granulocytic leukemia. Here, likewise, the cellular accumulations were of the same constitution as in the marrow, with absence of erythropoiesis and megakaryocytes. The conclusion that an organ was involved in the leukemic process depended upon the presence of leukemic cells outside the blood vessels or within markedly dilated capillaries containing few or no erythrocytes. Extravascular

TABLE IV
Clinical Data

Category	Necropsy number	Age	Sex	Known duration	Estimated duration	X-ray irradiation	Spleen			Liver			Lymph nodes			Bone pain	Basal metabolic rate
							Tenderness	Size	Duration*	Tenderness	Size	Duration*	Tenderness	Size	Duration*		
Chronic granulocytic leukemia	285	62	M		15 mos.	0	0	1 f† below costal margin		0	1 f below costal margin		0	Normal		0	
	1816	32	F	7 days		0	0	9 cm. below costal margin	7 days	0	Not palpable		0	Shotty	Few days	0	
	3110	18	M	10 mos.	14 mos.	750 r.	+	2 f below costal margin	12 mos.	0	Normal		0	Shotty	12 mos.	0	+16
	3538	45	F	22 hrs.		0	0	Not palpable		0	Not palpable		0	Not palpable		0	
	3677	68	M			725 r.	+	4 cm. below costal margin	1 day	0	? Palpable	1 day	0	Normal		0	
	5288	47	M	17 mos.	20 mos.	625 r.	+	At symphysis	17+ mos.	0	3 cm. below costal margin	17+ mos.	0	Not palpable		0	
	5380	51	M	10 mos.	14 mos.	150 r.	+	3 f below costal margin	1 mo.	0	Not palpable		0	Shotty		0	+54
	5818	58	F	4 mos.	19 mos.	0	0	Tip palpable	4 mos.	0	4 cm. below costal margin	8 mos.	0	Small	2 mos.	0	
Panmyelosis without myelofibrosis	474	55	F	24 mos.	24+ mos.	+	+	3 cm. above iliac crest	24 mos.	0	11 cm. below costal margin		0	Shotty	1 wk.	0	
	2231	25	F	21 mos.	36 mos.	200 r.	0	16 cm. below costal margin	20 mos.	0	Not palpable		0	0		0	+34
	2848	41	M	18 mos.	38 mos.	2,625 r.	+	Into pelvis	18 mos.	0	Palpable	18 mos.	0	0		0	+47
Panmyelosis with myelofibrosis	3125	64	F	76 mos.	112 mos.	3,508 r.	+	Into pelvis	76 mos.	0	Enlarged	72 mos.	0	0		0	+35
	3555	42	F	15 mos.	25 mos.	480 r.	+	Into pelvis	15 mos.	0	2 f below costal margin	3 mos.	0	0		0	+19
	4964	42	F	15 mos.	?	1,110 r.	+	Into pelvis	15 mos.	+	4 cm. below costal margin		0	0		0	+68
	5335	53	M	7 mos.	11+ mos.	0	+	16 cm. below costal margin	11 mos.	+	16 cm. below costal margin	11 mos.	0	0		0	+35
Transition	2576	42	F	26 mos.	36 mos.	400 r.	+	13 cm. below costal margin	30 mos.	0	5 cm. below costal margin	22 mos.	0	0		0	+38
	3360	36	F	3 mos.	3+ mos.	0	+	2 f below umbilicus	2+ mos.		2 f below costal margin	1+ mos.	0	0		0	+52
	4326	47	F	12 mos.	19 mos.	1,600 r.	+	At iliac crest	5 mos.	0	Not enlarged		0	0		0	+24
	5861	63	M	12 mos.	20 mos.	375 r.	+	2 cm. above iliac crest	13 mos.	0	3 f below costal margin	13 mos.	0	0.5–1.0 cm.	3 mos.	+	+14
Acute granulocytic leukemia	3081	20	M	9 mos.	33 mos.	450 r.	+	9 cm. below costal margin	9 mos.	0	4 f below costal margin	9 mos.	+	Increased	3 wks.	0	+50
	3745	19	M	1 wk.	12 mos.	0	0	3 f below costal margin	1 wk.	0	0		+	Increased	5 mos.	0	
	4663	35	M	2 mos.	2 yrs.	0	0	? Palpable		0	Enlarged		0	Not enlarged			

* Duration of increased size.
† Fingerbreadth(s).

leukocytic accumulations which were only hemorrhagic or exudative had to be recognized, for, as Table III shows, 89.5 per cent of the 19 cases showed a peripheral "leukemia." This situation readily leads to post-mortem clot formation resembling invasion of organs, or, in the event of infection, to foci of inflammatory exudate which may be misinterpreted as leukemic.

In summary, of 19 cases that had been labeled "chronic myelogenous leukemia," only 8 showed a pattern justifying the diagnosis of chronic granulocytic leukemia. This consisted in a relatively uniform hyperplasia of granulocytes in the bone marrow with reduction or even disappearance of other hematopoietic elements; partial replacement of the splenic parenchyma by leukemic cells of the same kind; involvement of the liver sinusoids but, even more prominently, of the portal fields; alteration of the lymph nodes by granulocytic proliferation; and, frequently, massive proliferation in other viscera not commonly associated with extensive hematopoiesis.

It is opportune, at this point, to indicate that case 3677 was an example of polycythemia vera of 3½ years' duration. The manifestations of polycythemia gradually disappeared and were eventually replaced by the hematologic characteristics of chronic granulocytic leukemia, confirmed by necropsy.

Acute Granulocytic Leukemia

The myeloblast dominates the proliferations in acute granulocytic leukemia. In these cases the pattern of involvement was identical with that described for chronic granulocytic leukemia. The only apparent difference was the presence of the myeloblast, rather than the myelocyte and its descendants, in the proliferations. The absence of differentiated granulocytes resulted in a more nearly homogeneous picture. The reduction to disappearance of other marrow elements probably accounted for anemia, thrombocytopenia, and purpura. The prominent involvement of hepatic portal fields was likewise similar to that of the first group, as was the splenic involvement. The lymph nodes were markedly enlarged and their normal architecture largely erased by homogeneous fields of leukemic cells; megakaryopoiesis and erythropoiesis were rare to absent. As in chronic granulocytic leukemia, involvement of other viscera was characteristic and even more massive.

These two groups, chronic and acute granulocytic leukemia, are the prototypes of "myelogenous leukemia," as generally taught.

Panmyelosis

The 7 examples of panmyelosis, constituting 36.8 per cent of the cases originally diagnosed as "chronic myelogenous leukemia," show a pattern of development and a basic composition which makes it quite possible to separate them from chronic granulocytic leukemia. Despite the occurrence of transition cases, which will be discussed, the basic histopathologic pattern is epitomized by the pleomorphism of the proliferations in all sites.

In the 3 cases without myelofibrosis (cases 474, 2231, and 2848) there was a diffuse hyperplasia of the *bone marrow* characterized by the presence of numerous megakaryocytes, granulocytic production of all types, and foci of erythropoiesis (Table II). Occasionally the megakaryocytes showed phagocytosis of leukocytes. Because of their striking form these cells were often conspicuous, but the pleomorphic hyperplasia of all normal elements (Fig. 5) was the significant difference from the homogeneous proliferation of granulocytes seen in chronic granulocytic leukemia.

The 4 cases with myelofibrosis differed from these 3 cases only in the appearance of the bone marrow. In cases 3125 and 3555 the picture just described was altered only by the addition of reticulum cells associated with foci in which an inconspicuous reticulin network appeared within the hyperplastic marrow (Figs. 9 and 10). In cases 4964 and 5335 this process was accentuated so that a denser network of fibers produced typical examples of myelofibrosis. The marrow survey in case 4964 was incomplete, since only several vertebrae were available for study. However, they showed a diffuse reticulin net woven through a still hyperplastic marrow (Figs. 11, 12, and 13). When myelofibrosis was more severe, scattered lymphocytes and plasma cells accompanied the granulocytes, nucleated red cells, and megakaryocytes. Also, the granulocytes and nucleated red cells lying amidst the fibers often were no longer organized into hematopoietic islands. Then hematopoietic cells and islands appeared conspicuously within widely dilated sinuses (Fig. 11). Reference to Table I shows that in the marrow of case 5335, which exhibited the most advanced fibrosis, there was a distinct decrease in hematopoietic cells (Fig. 14). Fibrosis of varying degree was also a feature of the 4 transition cases (Table I).

Osteosclerosis, involving the formation of new bone, was found in some of these cases. In 5 (Table I) osteogenesis was seen in all bones studied, differing in degree, however, from bone to bone within each

case. When it occurred, new bone formation was an accompaniment of fibroblastic activity. Medullary trabeculae were irregularly thickened by appositional membranous bone formation, and the endosteal layer of the cortex participated focally in this process (Fig. 15). However, concomitant hyperplasia of the marrow sometimes expanded the medullary cavities to produce cortical osteoporosis in other areas (case 5861).

The spleens, livers, lymph nodes, and other viscera of both subgroups of panmyelosis will be described together since they were identical.

The *spleens* were large, as the weights and the clinical measurements before roentgen irradiation indicate. The largest weighed 4,200 gm.; the average weight was 2329 gm. (Table IV). In the largest, the follicles were no longer present; in the smaller they were still visible. Hematopoiesis stood in the foreground, occurring both intra- and extra-sinusoidally (Fig. 6). Numerous foci of granulo- and erythro-cytogenesis were present, each containing normal appearing representatives at all stages of development. Outstanding by virtue of size and frequently number were the megakaryocytes. As in the marrow, some megakaryocytes displayed active phagocytosis. In short, the hematopoiesis is identical with that described in the marrow, and to carry the comparison further, there was also fibrosis in Billroth's cords in a few instances. The appearance is distinctly unlike that of acute or chronic granulocytic leukemia. Infarcts may be present or absent and are of no value in distinguishing between examples of granulocytic leukemia and panmyelosis.

The *livers* were all enlarged but varied markedly in size; their weights ranged from 1,800 to 6,000 gm. The cellular composition of the proliferations was pleomorphic and like that of normal marrow. Megakaryocytes were abundant among the granulocytic and erythropoietic foci. Of great significance was the distribution of the hematopoietic cells between the sinusoids and the portal fields. The sinusoids were the sites of marrow cell production, but there was little or no participation of the portal fields even when the sinusoids were maximally involved (Fig. 7). This distribution follows that of physiologic hepatic hematopoiesis as seen in the fetus, in the newborn infant, and in the adult with extramedullary non-leukemic hematopoiesis.[7] In contrast, it will be recalled that in chronic and acute granulocytic leukemia the portal fields rather than the sinusoids were most heavily involved. In areas, veritable lakes of hematopoiesis had caused adjacent and traversing liver cords to undergo atrophy and finally to dis-

appear (Fig. 16). However, in some instances, hematopoiesis was almost absent except for megakaryocytes which lay scattered about in the sinusoids (Fig. 17), curiously reminiscent of megakaryocytic embolization of the lung.

The *lymph nodes* were invariably small. In one case, no. 2848, they were so inconspicuous as to have escaped the prosector's attention. Involvement was constant, however, in the para-aortic and variable in the peripheral groups. Here, as elsewhere, the megakaryocyte, the hallmark of panmyelosis, first caught the eye (Fig. 8). Erythro- and granulo-poiesis were apparent, with the former less prominent. The architecture of the lymph nodes was maintained except in case 5861 in which focal fibrosis was found (Fig. 18). As in the spleen, intra- and extra-sinusoidal hematopoiesis, and even fibrosis, imitated the marrow in all details.

The other viscera may be entirely uninvolved or only minimally so. Comparison of the granulocytic leukemia groups with the panmyelosis groups effectively underscores this difference. In panmyelosis, if viscera other than liver or spleen are involved, it is to a minimal extent, entirely disproportionate to the degree of hepato-lienal enlargement. This general rule was violated only in case 5861 (Table I). In the microscopic foci found, the cells were of various kinds just as in liver and spleen. Megakaryocytes, however, were not commonly seen. These large cells are first to catch the eye, but they are the least numerous of the hematopoietic cells. Hence, they would be rare or absent in small foci of hematopoiesis.

In summary, 7 of our 19 cases originally diagnosed "chronic myelogenous leukemia" were instances of what we have called panmyelosis. In these cases pleomorphic proliferations were found in the bone marrow, spleen, liver, and lymph nodes but rarely in other organs and tissues. Granulocytes, erythrocytes, and megakaryocytes were present in varying combination. In some cases, reticulum cells and a fibrous reticulin network were added to the basic pattern. When present, fibrous tissue usually was most conspicuous in the bone marrow but was observed also in the spleen and in lymph nodes. Table II summarizes the morphologic differences between acute and chronic granulocytic leukemia and panmyelosis.

The transition cases were segregated because they showed the following mixed features:

Case 2576 was in all respects, save one, an excellent example of panmyelosis. The exception lay in the structure of the mesenteric lymph nodes. These showed effacement of normal structure and re-

placement by granulocytes characteristic of chronic granulocytic leukemia.

Cases 3360 and 5861 were examples of panmyelosis with myelofibrosis and osteosclerosis associated with acute (myeloblastic) granulocytic leukemia. Certain special features of case 5861 set it somewhat apart from the other cases of panmyelosis. The foci of pleomorphic hematopoiesis (panmyelosis), aside from the acute myeloblastic proliferations, more nearly followed the pattern of chronic granulocytic leukemia. The kidneys (Fig. 19), lymph nodes (Fig. 18), and retroperitoneal fatty reticulum were extensively involved; the pericardium, adrenal glands, pancreas, thyroid gland, and peribronchial structures participated only minimally in the process. The bone marrow was so hyperplastic that the ribs, vertebrae, and sternum were expanded; yet there was focal myelofibrosis and osteosclerosis. Fibrosis in the spleen and in lymph nodes demonstrated the fact that in panmyelosis the formation of reticulin and collagen fibers is not restricted to the bone marrow. As in the other cases of panmyelosis, the hepatic sinuses contained more hematopoietic cells than the portal fields. At all of these sites clusters of myeloblasts were found; they even appeared in areas of fibrous marrow.

Case 4326 anatomically was one of panmyelosis with myelofibrosis and sclerosis associated with reticulo-endotheliosis, but hematologically it was considered to be a case of acute myeloblastic leukemia.

The significance of these atypical cases will be discussed.

The Nature and Pathogenesis of Panmyelosis

Our observations indicate that, regardless of clinical course or hematologic considerations, the pathologist can in most cases distinguish panmyelosis from the granulocytic leukemias. The statement supports those already recorded in the literature by numerous observers.[8-11] However, to our knowledge the study presented in this paper represents the first pathologic-anatomical approach to this problem of differentiation through an analysis of a group of cases routinely considered to be instances of "chronic myelogenous leukemia" by pathologists and clinicians whose competence ordinarily would hardly be questioned. This approach is necessary if one is to estimate the prevalence of panmyelosis, for this cannot be determined from the literature.

The pertinent literature, which has recently been reviewed,[12] contains a number of instances of panmyelosis reported under a variety of names. There are numerous reports of single cases, many comprehensively presented. However, these cases often have been considered

without reference to the larger problem of the myeloses. Apparently this has resulted from a desire on the part of the contributor to point up certain unusual hematologic, clinical, or pathologic features of the case. Even when groups of cases have been studied[8,12] no data relating to frequency have been recorded. Of special interest is the fact that, despite the large number of cases in some reviews of "leukemia," the authors have found no instances of panmyelosis, either with or without myelofibrosis.[13] A survey of our cases of "leukemia" might well have had the same result had the first recorded pathologic diagnosis alone been utilized.

It seems, then, that panmyelosis is not generally recognized, and surely it is not widely accepted as an entity. The cases presenting the least resemblance to chronic granulocytic leukemia have appeared in the literature under titles which emphasize one or another hematologic peculiarity; for example, "myelofibrosis," "nonleukemic myelosis," "megakaryocytic leukemia." The cases closely resembling granulocytic leukemia generally appear to have justified no particular differentiative diagnostic attention, perhaps because of the generally ineffective therapy in all leukemias. Apparently for this reason such cases rarely have been reported. It is therefore not surprising that there has been no widespread interest in the matter of critical differentiation of the cases of "myelogenous leukemia."

There are two opinions as to the wisdom of separating panmyelosis from chronic granulocytic leukemia. Those who hold that no separation is justified, the "unitarians," are represented by Heller, Lewisohn, and Palin[12]; the "dualists," who hold that panmyelosis and granulocytic leukemia are distinct entities, are represented by Vaughan,[8] Hickling,[9] Jackson, Parker, and Lemon,[11] Rosenthal and Erf,[14] and Churg and Wachstein.[15] Both groups reach their conclusions after a point-by-point examination of morphologic, hematologic, and clinical data such as are recorded in our Tables II, III, and IV. The two viewpoints cannot be reconciled easily since they are the results of different attitudes toward the data. On the whole, those who would not recognize, or who at least would minimize, the differences between panmyelosis and chronic granulocytic leukemia are today in the ascendancy. This is clearly indicated by the treatment of the subject in current textbooks of pathology and hematology.

In contrast, there is general acceptance of the concept of "myelophthisic anemia" (erythroleukemia) associated with any process which replaces most of the marrow, *e.g.*, metastatic carcinoma and generalized xanthomatosis. In some, but not all, cases of myelophthisic

anemia extramedullary blood formation is found especially in the spleen and the liver. This extramedullary hematopoiesis is usually considered to be "compensatory" myeloid metaplasia, the result of bone marrow replacement. In these cases the leukocyte count may be low, or very high: when high it frequently leads to a mistaken diagnosis of chronic granulocytic leukemia; when low, to that of subleukemic granulocytic leukemia. Similarly, in cases of panmyelosis in which the bone marrow is fibrotic or sclerotic, hematopoiesis in the spleen, liver, and elsewhere is believed to be "compensatory" to its reduction in the bone. Our cases do not support this interpretation. Hematopoiesis may cause the spleen and the liver to be just as large when the bone marrow is pleomorphically hyperplastic as when it is fibrotic. Furthermore, fiber formation is not restricted to the marrow; it is found also in the spleen and the lymph nodes. This would suggest that the formation of fibers is just another aspect of differentiation in this generalized, pleomorphic proliferative reaction of the multipotent mesenchymal tissue forming the marrow.

In cases of panmyelosis described by Vaughan[8] there was a hyperplastic marrow containing numerous foci of erythropoiesis associated with persistent anemia characterized by circulating nucleated red cells. As an explanation of this, she suggested a disturbance of maturation and a disruption of the mechanism of red and white cell delivery caused by the absence of an unknown factor, a situation analogous to that in pernicious anemia. Although this is an interesting suggestion, to us it would not appear necessary to postulate a deficiency factor in the pathogenesis of this condition, since a substance capable of producing every feature of panmyelosis in man, from hyperplasia of the marrow to myelofibrosis, is well known. This substance is benzol (benzene).[16] Furthermore, radium probably produces the same effects. The studies of Mallory, Gall, and Brickley[16] are revealing in this connection. They described 14 necropsies performed on individuals exposed to benzol, 12 of which revealed all the characteristics of panmyelosis with and without myelofibrosis. Their illustrated lesions are identical with those in our cases and in the literature, indicating that extrinsic benzol intoxication is at least one cause of panmyelosis. Also, it probably is significant that in about half of the cases reported by Jackson, Parker, and Lemon[11] the patients had been exposed to benzene or related compounds.[17]

Martland[18] described the early marrow reaction in cases of accidental, fatal, chronic ingestion of radium salts as hyperplasia involving both myeloid and erythroid elements. Megakaryocytes usually

were abundant. This was considered by Martland to be "irritative." It was followed in some individuals by myelofibrosis (radiation osteitis), in some instances requiring years for development. Mallory and his co-workers[16] were impressed by the basic similarity of their cases to those described by Martland.

It has been shown experimentally that a variety of agents will produce myelofibrosis and sclerosis with either hyperplasia or aplasia of blood-forming elements. Firket and Campos,[19] by injecting saponin into rabbits, produced marked megakaryocytosis of the spleen and liver, as well as hyperplasia of all elements of the bone marrow, including the megakaryocytes. Silberberg and Silberberg[20] discovered that guinea-pigs given large doses of estrogen eventually develop myelofibrosis and sclerosis, accompanied by an increase of marrow megakaryocytes. These experimental observations (there are many others) together with those in man made by Mallory, Gall, and Brickley[16] suggest that many agents may produce panmyelosis comparable to that in our patients.

The cause of panmyelosis with or without myelofibrosis in man is unknown except for chronic benzol poisoning and possibly the ingestion of radium. Nevertheless, attempts to identify extrinsic etiologic agents would seem important if cases of panmyelosis actually do constitute about one third of all examples of chronic myelosis, as indicated by our experience and that of Block and Jacobson.[21] Table I in their report shows that among 55 patients undergoing splenic puncture there were 9 cases of "myelogenous leukemia" and 7 cases of "myeloid metaplasia." Furthermore, they wrote: "In the past eighteen months 8 cases of myeloid metaplasia have been discovered in a group of patients referred with the diagnosis of leukemia . . . . in none would the disease have been diagnosed correctly without splenic biopsy." In short, the more extensive the clinical investigation, the more frequent the diagnosis of panmyelosis.

Recently Wyatt and Sommers[22] published a highly stimulating study of 30 cases of myelosclerosis and extramedullary hematopoiesis in which they emphasized "chronic marrow failure" as the basic pathogenic mechanism. Their cases appear to be identical with our cases of panmyelosis. These authors suggested "a working hypothesis of the pathogenesis of" the disease. They postulated a failure of the liver to conjugate and excrete exogenous benzene and related aromatic compounds and/or endogenous steroidal phenolic compounds. These substances they considered to be toxic for the "partly mature hematopoietic cells" and they suggested that this might account for the

myeloid necrobiosis found in their cases. They suggested further that "protein breakdown products may then form trophic stimuli to hyperplasia of the surviving cells and extramedullary hematopoiesis." While we find ourselves in substantial agreement with their description of most of the lesions, we have not observed necrobiosis of the bone marrow in our cases.

Transition cases combining the various features of granulocytic leukemia and panmyelosis suggest that the two processes are allied. However, such cases present no difficulties to a dualistic interpretation if one recalls, as did Vaughan,[8] that the primitive mesenchyme of bone marrow may elaborate, under different conditions, reticulin and collagen, bone or hematopoietic corpuscles of all types. The well known conversion of polycythemia vera to either granulocytic leukemia (case 3677) or aplastic anemia with myelofibrosis (panmyelosis?), of panmyelosis with myelofibrosis to acute granulocytic leukemia, of chronic to acute granulocytic leukemia is good evidence that all forms of myelosis are fundamentally related. Knowledge of the relationships of the acute and chronic granulocytic leukemias and polycythemia vera does not prevent their distinction clinically or pathologically, whenever possible, for reasons of prognosis and treatment. The panmyeloses should be separated from the granulocytic leukemias for identical reasons. More certain identification of entities and understanding of transitions, when they occur, await further knowledge of response of the reticulo-endothelium to various agents and stimuli, some of which surely are not yet recognized.

The Effects of Irradiation and of Splenectomy in the Myeloses

The effects of therapy upon the various hematopoietic centers very likely are responsible for some of the difficulty in post-mortem classification of the myeloses. The similar effects of nitrogen mustard, urethane, and external or internal radiation have been pointed out repeatedly.[3,4,6] It seems clear that treatment may profoundly alter the appearance of the leukemic lesions. The rabbit's spleen 8 days after 600 to 800 r. spray radiation shows megakaryocytosis, erythropoiesis, and myelopoiesis highly reminiscent of the human spleen in panmyelosis. Indeed, the process is panmyelosis. The same process is concurrently visible in the bone marrow. It is conceivable that irradiation of the hematopoietic tissues in a case of chronic or acute granulocytic leukemia could achieve a similar result, followed by a gradual return to the original pathologic state. Should the patient die during

the early period of therapeutic remission, the tissues might present the appearance of panmyelosis. However, analysis of our cases (Table IV) clearly shows that this could not have been a significant factor. Each category, except that of non-myelofibrotic panmyelosis, includes at least one patient whose hematopoietic tissues were never treated with roentgen irradiation (cases 285, 1816, 3538, 5818, 5335, 3360, 3745, 4663). In the category of non-fibrotic panmyelosis, case 474 was given 100 r. to the area of the spleen just 8½ hours ante mortem. No change as widespread or as characteristic as that found could have been effected by that radiation in so short a time. Furthermore, Hickling[9] listed cases of panmyelosis reported before 1903, when roentgen irradiation was first used to treat leukemia. It would appear, therefore, that the characteristic extramedullary and medullary hematopoiesis of panmyelosis with or without fibrosis is not an artefact of treated granulocytic leukemia but a spontaneous manifestation of diseased hematopoietic mesenchyme.

It has been stated[9] and restated[11] that splenectomy is contraindicated in panmyelosis. Likewise, irradiation[21] of the spleen has been considered a functional splenectomy and, therefore, unwise treatment. It is assumed that the extramedullary blood production may be largely concentrated in the spleen and that its elimination may precipitate leukopenia, thrombocytopenic purpura, or aplastic anemia leading rapidly to the death of the patient. The first hint that splenectomy might be a fatal maneuver in panmyelosis was advanced by Hickling,[9] who, from his review of the literature and of his own cases, reported 27 examples of splenectomy, followed in 15 instances by death within the first postoperative week. No careful postoperative hematologic studies were made, and, indeed, in other cases in which such studies are reported no unusual manifestations were noted. Yet examination of the pertinent literature reveals many instances of splenectomy, and, in our cases, repeated splenic irradiation—as many as 24 times in a single individual and frequently as intense as 300 r. (case 3125)—followed by survival for years.

The preceding discussion is not to be interpreted as indicating that splenectomy may not be without unwelcome sequelae. It is possible that in myelofibrotic panmyelosis the major source of blood production may be the spleen and, in such an instance, splenectomy would be contraindicated. The existing conditions can be determined clinically by a series of biopsies of bone, splenic aspirations, and needle biopsies of the liver. However, in our experience most instances of panmyelosis are associated with hyperplasia and not hypoplasia of the marrow.

Obviously, in such hyperplastic cases, the loss of the spleen or of its function may result in either a transient reduction in the number of circulating white and red cells or no hematologic effect. Even when myelofibrosis is already present it must be realized that the marrow may still be hyperplastic. Some cases,[9] although few in number, reveal that splenectomy, contrary to the usual suppositions, may be followed by the conversion of subleukemic (non-leukemic myelosis) panmyelosis to a frank leukemic picture or, if a mild leukemic hemogram existed, by a rising white count. These cases would seem to indicate that the large spleen of panmyelosis may on occasion exert an inhibitory effect upon the number of circulating leukocytes. Whether this hematologic conversion is in itself deleterious is unknown. The erythrocyte and platelet counts remained unchanged. Too few well studied cases of splenectomized panmyelosis have been reported to justify generalizations.

It appears from our data (Table V) that irradiation of the spleen may be quite helpful (cases 3125, 2848). However, the value of such data as recorded is limited by the small number of individuals in even the larger categories, and the lack of uniformity of treatment. Since so little is known about the reaction of individuals with panmyelosis to roentgen irradiation, the data are published so that they may be consolidated by others with their own. In this way the number of cases necessary for the extraction of valid generalizations may be obtained.

Case 3125, an instance of panmyelosis with minimal myelofibrosis (Figs. 9 and 10), received, in all, twenty-four separate splenic irradiations, usually from 150 to 200 r. at a time. This was carried out over a period of 4⅓ years. The response was always a reduction in the circulating leukocytes, a rise in erythrocytes and hemoglobin, with no significant change in the platelet count. Of some importance to the question of ultimate medical control of panmyelosis is the fact that this patient responded as well to Fowler's solution as she did to x-ray; and, indeed, was carried for the last year of her life on that medication. The patient developed hypertension and eventually died in uremia; the necropsy revealed arterio- and arteriolo-nephrosclerosis. Seemingly, the patient's life was not shortened by the presence of the hematologic disease which had probably existed 10 or more years and was diagnosed for 7 years as "chronic myelogenous leukemia."

Case 4964, an example of more advanced myelofibrosis (Figs. 11, 12, and 13), presented another instance of the relative resistance of the hematopoietic system in this condition to massive roentgen irradiation of the spleen. The schedule of treatment follows:

1–22–47	50 r. Spleen	1–28–47	20 r. Spray
1–23–47	100 r. Spleen	1–29–47	200 r. Spleen
1–24–47	20 r. Spray	1–30–47	20 r Spleen
1–25–47	200 r. Spleen	2–13–47	150 r. Spleen
1–27–47	200 r. Spleen	2–14–47	150 r. Spleen

TABLE V

Roentgen Therapy

Category	Case no.	Total radiation therapy	Time span	Dose last therapy and site	Duration	Time ante mortem	Hematologic response	Cause of death
Panmyelosis without myelofibrosis	474	X-ray, unknown	2 mos.	100 r., spleen	1 day	8½ hrs.	No data	?
	2231	X-ray, 200 r.	6 days	200 r., spray	6 days	9 days	Inadequate data	Bacteremia
	2848	X-ray, 2,625 r.	13 mos.	300 r., spleen	2 days	2 days	No data	
Panmyelosis with myelofibrosis	3125	X-ray, 3,508 r.	4⅛ yrs.	400 r., spleen	2 days	1 yr.	W, decreased E, increased P, no change	Uremia
	3555	X-ray, 480 r.	11 mos.	50 r., spray	1 day	4 mos.	W, E, and P, decreased	Cerebral hemorrhage
	4964	X-ray, 1,110+ r.	?7 mos.	1,070 r., spleen; 40 r., spray (26.0 mg. nitrogen mustard)	22 days	4 wks. (7 days)	W, increased P, no change	Subdural hemorrhage (fall)
	5335	X-ray, 700 r.	5 days	700 r., aortic valve	5 days	4 mos.	W, E, and P, no change	Heart failure, aortic stenosis

r. = roentgens; W = leukocytes; E = erythrocytes; P = platelets.

The hematologic response is recorded below:

	1–13–47	1–29–47	2–5–47	3–6–47
Hemoglobin	5.4 gm.	8.8 gm.	6.9 gm.	8.2 gm.
Platelets	350,000	"adequate"	200,000	200,000
Leukocytes	62,000	24,000	9,300	102,000

Despite heavy splenic radiation, there was no aplastic anemia, thrombocytopenia, nor leukopenia, but eventually an accentuation of the leukemic aspect of the myelofibrotic panmyelosis.

Case 4326, an instance of severe generalized myelofibrosis with reticulo-endotheliosis (hematologically, myeloblastic crisis), presented an astonishing sequel to the following irradiation:

12–28–44	200 r. Spleen
12–29–44	200 r. Spleen
	200 r. Shoulder
12–30–44	200 r. Spleen
	200 r. Shoulder
1–2–45	150 r. Spleen

The patient was discharged, then readmitted on February 8, 1945, having done poorly at home since his irradiation, and died on March 5, 1945.

The hematologic course is detailed as follows:

	12–28–44	12–30–44	2–9–45	2–27–45
Hemoglobin	9.7 gm.	7.9 gm.	7.4 gm.	8.8 gm.
Platelets	300,000			80,000
Leukocytes	206,000	142,000	12,000	153,000
Polymorphonuclear cells	59%		10%	0%
Myelocytes	38%		1%	0%
Myeloblasts	3%		84%	99%
Lymphocytes			5%	1%
Remarks	Occasional nucleated red blood cells present			

About 1 month after treatment a major change in the nature of the disease was manifest. The diagnosis had been "chronic myelogenous leukemia," but by February 9, 1945, it was changed to "acute granulocytic leukemia" showing a fulminating course and terminating in thrombocytopenic purpura, agranulocytosis, and bacteremia. It is futile, with present-day knowledge, to discuss at length cause and effect in this case. Suffice it to record what may have been a remarkable coincidence or an upset by the radiant energy of a delicate metabolic balance in the hematopoietic system. This effect was not limited to the spleen, which, except for the shoulder, alone was irradiated, but was manifested throughout the body. Thrombocytopenia and agranulocytosis were not due to an absence, relative or absolute, of the respective precursors in the spleen, or a "burning-out" of hematopoietic tissue, but rather to a reorientation of the primitive hematopoietic mesenchyme.

Case 5861 is in some respects similar to the preceding case. The patient was suffering from a myelofibrotic panmyelosis with a leukemic hemogram complicated by severe mitral insufficiency. He was treated with x-ray irradiation as follows:

6–28–49	100 r. Spleen
6–29–49	100 r. Spleen
6–30–49	25 r. Spleen
7–15–49	75 r. Spleen
7–22–49	75 r. Spleen

The hematologic reflection of the therapy and the course of the leukemic aspects of the disease are recorded in the following peripheral blood counts:

	8–10–48	9–3–48	6–24–49	7–14–49	7–29–49
Red blood cells	2.75 million	3.27 million	2.5 million	2.06 million	1.75 million
Platelets	700,000	3,000,000	"adequate"		"adequate"
Leukocytes	15,700	36,000	47,000	22,850	38,000
Myeloblasts	4%	4%	0%		64.0%

In Table I, the preceding cases, 4326 and 5861, are listed as transitions from panmyelosis to acute granulocytic leukemia. To what extent the roentgen therapy was responsible for the reorientation toward "myeloblastic leukemia" of what had previously been considered characteristic "chronic myelogenous leukemia" is again unknown.

It is conceded that conversion of "chronic myelogenous leukemia'" to "myeloblastic leukemia" may occur without the agency of roentgen rays or other known treatment and so it may be in the preceding cases of panmyelosis; yet the close temporal proximity in the 2 cases, 4326 and 5861, at least justifies suspicion that x-ray energy may have been responsible.

Case 5288, an example of chronic granulocytic leukemia, revealed an interesting reaction to the following roentgen irradiation:

1–21–48	100 r. Spleen
1–22–48	100 r. Spleen
1–23–48	75 r. Spleen

Results of examination of the blood before and after irradiation were:

	1–19–48	1–26–48	2–17–48
Hemoglobin	12.8 gm.	6.7 gm.	6.5 gm.
Platelets	108,000	37,000	5,000
Leukocytes	47,000	31,500	62,000

The patient died on February 18, 1948, following a severe gastro-intestinal hemorrhage.

It is an important aspect of this case that the spleen, 3½ weeks postirradiation, was a mass of necrotic tissue. In contrast, the spleen of case 4964 (myelofibrotic panmyelosis) showed no evidence of necrosis despite receiving 300 r. 4 weeks prior to necropsy, which was 25 r. more than in the present case. The effect of irradiation was obvious in the precipitous fall of platelets and hemoglobin, the latter a consequence of thrombocytopenic hemorrhage from the gastro-intestinal tract. The low platelet count of 108,000 prior to x-ray treatment already indicated the precarious position of the megakaryocytes. Roentgen irradiation of the spleen alone was somehow sufficient to upset whatever equilibrium existed and thus to bring on disaster.

The appearance of thrombocytopenia following local roentgen irradiation alone has been observed by Mossberg.[23] The close sequence of irradiation and thrombocytopenia in patients with previously normal numbers of platelets led him to conclude that localized roentgen irradiation may precipitate thrombocytopenic purpura. The existence of this complication of local irradiation is not widely appreciated.

No comments are made on several of our cases, since insufficient data are available (474, 2848).

In summary, two conclusions may be drawn from a study of roentgen ray therapy of the cases of hyperplastic and myelofibrotic pan-

myelosis: (1) Irradiation of the spleen in panmyelosis does not necessarily, or even probably, lead to a hematologic crisis in the sense of aplastic anemia, leukopenia, or thrombocytopenia. In fact, irradiation may in some individuals be quite useful. (2) The effects of irradiation are unpredictable, indicating individual biochemical differences, despite basically similar morphologic features.

The Dissimilarity of Chronic Granulocytic Leukemia and Panmyelosis with Respect to Clinical Character and Duration

It has oeen demonstrated in the preceding pages that in the Duke Hospital, of 19 individuals diagnosed clinically and originally at necropsy as "chronic myelogenous leukemia," subsequent study of necropsy material revealed in 7 cases the anatomical characteristics of panmyelosis. The average duration of life from the estimated onset of the disease (clinical history) in 6 of these 7 patients is 41 months, varying from 11 to 112 months. In case 5335, with the shortest estimated duration (over 11 months), the disease was first recognized when the spleen was palpable 16 cm. below the costal margin. The same is true of cases 474 and 4964. Presumably, these patients had had panmyelosis for several years when the condition was detected. It is apparent from this that survival from onset in this disease extends well beyond the known duration.

In contrast to the estimated minimal average survival of 41 months in 6 instances of panmyelosis, the estimated survival in 5 cases of chronic granulocytic leukemia averaged 16.4 months (14 to 20 months). It becomes apparent that of the cases originally called "chronic myelogenous leukemia" those which were examples of panmyelosis presented a more favorable prognosis.

The reports already cited indicate that long-lived patients with "chronic myelogenous leukemias" often reveal at necropsy more or less typical panmyelosis, with or without myelofibrosis. Some of these individuals die not of their myelosis but with it. This relationship in our cases 3125 and 5861 has already been discussed. In case 5335 death resulted from protracted cardiac failure caused by extreme calcific aortic stenosis. In case 4964 death occurred after a fall which produced a subdural hematoma. Vaughan[8] reported one of her cases as having a duration of 10 years; Jackson, Parker, and Lemon[11] described a case of 19 years' duration. One of the most important causes of death is the inanition which superficially resembles that accompanying advanced cancer. This arises in part from reduced dietary

intake due to an impaired appetite and in part from the increased metabolic rate which characterizes most cases of myelosis.

The elevated metabolic rates in these cases are listed in Table IV. The average basal metabolic rate for 6 instances of panmyelosis was plus 39.7 per cent. The lowest was plus 19 per cent, the highest 68 per cent. Here, as with the problem of longevity, the figures must be interpreted. The basal metabolic rate stands in a direct relationship to the activity of the disease. Following successful irradiation, for example, when the number of leukocytes has diminished, that of the erythrocytes increased, and the patient is otherwise objectively and subjectively improved, the basal metabolic rate approaches normal values. When the patient eventually returns with recurrence of the disease, it is found that the basal metabolic rate has again risen. In selecting the representative figure for basal metabolic rate (Table IV), an attempt was made to find values corresponding to an active phase of the disease. However, in some instances only one test was recorded. The basal metabolic rate in case 3555, for example, was obtained during a post-therapeutic recession of the process and is not necessarily indicative of the patient's average rate.

The cause of the heightened metabolic rate probably is to be found in the activity of the hematopoietic system and not in the thyroid or pituitary glands; neither thiouracil nor iodine has any influence upon the basal metabolic rate when administered in full therapeutic doses. A few unpublished observations of our colleague Dr. J. D. Myers[24] are pertinent. He found that the total oxygen consumption in 2 cases of "chronic myelogenous leukemia" was increased 42 and 56 per cent over normal; and in one case of "aleukemic myelogenous leukemia," 20 per cent over normal. By catheterization of the hepatic vein an increase in splanchnic (largely hepatic, splenic, and intestinal) oxygen consumption was found. In the first 2 cases the spleens and livers were enlarged, and the increased splanchnic oxygen consumption accounted for about two thirds of the total increase in use of oxygen. In the third case the spleen and liver were not greatly enlarged, and the increased splanchnic oxygen consumption accounted for only about one third of the total increment. It seems likely that additional studies of this kind will demonstrate that the active hematopoietic tissues in the greatly enlarged livers and spleens account for much of the increased metabolic activity in panmyelosis. If this is true, the patient with chronic myelosis is subjected to the same metabolic stress as in experimental[25] and spontaneous[26] hyperthyroidism. A diet which is considered adequate for a normal individual may be entirely inade-

quate for these patients. This is true in a caloric as well as in a qualitative sense. Thus, the victims of the myeloses develop deficiencies which further impair that most potent weapon against excessive metabolic rate, the healthy appetite. The patients, as a consequence, decrease their intake and add to the metabolic stress producing inanition. In addition, a huge, tender spleen and often a large liver displace the intestines and diaphragm and produce a constant sensation of fullness. It is surprising that these patients survive as long as they do. It would appear that an unusually good diet may make possible a more successful adjustment to the demands of the high metabolic rate, as in hyperthyroidism, whereas a simple "normal" but, in reality, inadequate diet may appreciably shorten life.

In addition to the inanition and its consequences, the patients may succumb to the results of radiation therapy as is true for all the myeloses.

While the chief cause of death in panmyelosis appears to be inanition and its complications, in granulocytic leukemia it is outranked in importance by two other causes of death, namely, thrombocytopenic hemorrhage (Table III) and agranulocytic infection. Both are uncommon in untreated panmyelosis but common in untreated granulocytic leukemia, chronic or acute. Both may be induced by external or internal irradiation in diseased or normal people so that their occurrence in irradiated patients with panmyelosis is not always to be considered an aspect of the disease but may be a complication of treatment. The significance of this point is emphasized, but in our opinion unnecessarily so, by the interdiction of roentgen irradiation in the treatment of panmyelosis.

The hematologic data for the patients described are not sufficiently complete to justify a discussion. It seems, however, that in some cases of panmyelosis the hemogram may be indistinguishable from that of chronic granulocytic leukemia. As Block and Jacobson[21] have concluded, study of the myelogram in conjunction with splenic aspiration and possibly biopsy of the liver should leave little room for a mistaken diagnosis, provided that the entity of panmyelosis is kept in mind whenever an example of "chronic myelogenous leukemia" is encountered.

Summary and Conclusions

It appears important to make a diagnostic distinction between classical chronic granulocytic leukemia and a commonly occurring variant of this entity which has been recognized by a number of workers and described under a variety of names. This variant we have

called panmyelosis. This conclusion is the result of our restudy of all cases originally diagnosed as "chronic myelogenous leukemia" and necropsied at Duke Hospital between 1931 and May, 1950. Of 19 cases in all, 8 we have found to be justifiably diagnosed as chronic granulocytic leukemia; 7 are examples of panmyelosis. Four represent a transition from panmyelosis to chronic granulocytic leukemia.

The proliferations of the reticulo-endothelium, both medullary and extramedullary, are pleomorphic in the cases we have termed panmyelosis. The mixture of granulocytes, megakaryocytes, erythrocytes, reticulum cells, fibrocytes, and osteoblasts in various combinations stands in striking contrast to the nearly homogeneous granulocytic hyperplasia of the reticulo-endothelium in the cases of chronic granulocytic leukemia. The differences in cytologic composition and anatomical distribution of the hyperplastic tissue are summarized.

The etiology of the myeloses in man remains obscure. Knowledge is only fragmentary concerning agents and mechanisms that stimulate the reticulo-endothelium to proliferate and to differentiate in different directions. There is evidence that benzol and radium in man, and estrogens in the experimental animal, may provoke the reaction of panmyelosis. Metastatic carcinoma and other "replacements" of bone marrow sometimes may stimulate the extramedullary reticulo-endothelium in such a way that the resulting proliferation resembles the reaction in panmyelosis.

The transition of one myelosis into another indicates the extraordinary potency of the reticulo-endothelium to produce a wide range of end-products of differentiation. An understanding of these conversions awaits further knowledge of the mechanisms of normal differentiation. The possible rôle of therapeutic agents, especially x-rays, in altering the course of some of these differentiations has been considered.

The clinical duration is longer in cases of panmyelosis (from 11 to 112 months) than in the cases of chronic granulocytic leukemia (from 14 to 20 months). Inanition and cachexia are the chief cause of death in the former group whereas infection and hemorrhage are the most common fatal complications in the latter. Evidence does not warrant the allegation that splenectomy or splenic irradiation necessarily is followed by anemia, thrombocytopenia, and granulocytopenia in cases of panmyelosis.

An accurate evaluation of the effectiveness of the newer drugs in the treatment of the chronic myeloses would seem to rest on a more accurate anatomical assessment of the myelosis being treated, since the natural courses of the two major types, chronic granulocytic leukemia and panmyelosis, are often strikingly different.

REFERENCES

1. Arinkin, M. I. Die intravitale Untersuchungsmethodik des Knochenmarks. *Folia haemat.*, 1929, **38**, 233–240.
2. Neumann, E. Ueber die Bedeutung des Knochenmarkes für die Blutbildung. *Centralbl. f. d. med. Wissensch.*, 1868, **6**, 689. (Cited in Leitner, S. J. Bone Marrow Biopsy. Tr. by Britton, C. J. C., and Neumark, E. Grune & Stratton, New York, 1949, p. 1.)
3. Paterson, E., Thomas, I. A., Haddow, A., and Watkinson, J. M. Leukaemia treated with urethane compared with deep x-ray therapy. *Lancet*, 1946, **1**, 677–682.
4. Kindred, J. E. Histologic changes occurring in the hemopoietic organs of albino rats after single injections of 2-chloroethyl vesicants. *Arch. Path.*, 1947, **43**, 253–295.
5. Loge, J. P., and Rundles, R. W. Urethane (ethyl carbamate) therapy in multiple myeloma. *Blood*, 1949, **4**, 201–216.
6. Dameshek, W., Weisfuse, L., and Stein, T. Nitrogen mustard therapy in Hodgkin's disease. Analysis of fifty consecutive cases. *Blood*, 1949, **4**, 338–379.
7. Plum, C. M. Extramedullary blood production. *Blood*, 1949, **4**, 142–149.
8. Vaughan, J. M. Leuco-erythroblastic anaemia. *J. Path. & Bact.*, 1936, **42**, 541–564.
9. Hickling, R. A. Chronic non-leukaemic myelosis. *Quart. J. Med.*, 1937, **6**, 253–275.
10. Vaughan, J. M., and Harrison, C. V. Leuco-erythroblastic anaemia and myelosclerosis. *J. Path. & Bact.*, 1939, **48**, 339–352.
11. Jackson, H., Jr., Parker, F., Jr., and Lemon, H. M. Agnogenic myeloid metaplasia of the spleen. A syndrome simulating other more definite hematologic disorders. *New England J. Med.*, 1940, **222**, 985–994.
12. Heller, E. L., Lewisohn, M. G., and Palin, W. E. Aleukemic myelosis. Chronic nonleukemic myelosis, agnogenic myeloid metaplasia, osteosclerosis, leukoerythroblastic anemia, and synonymous designations. *Am. J. Path.*, 1947, **23**, 327–365.
13. Kirshbaum, J. D., and Preuss, F. S. Leukemia. A clinical and pathologic study of 123 fatal cases in a series of 14,400 necropsies. *Arch. Int. Med.*, 1943, **71**, 777–792.
14. Rosenthal, N., and Erf, L. A. Clinical observations on osteopetrosis and myelofibrosis. *Arch. Int. Med.*, 1943, **71**, 793–813.
15. Churg, J., and Wachstein, M. Osteosclerosis, myelofibrosis, and leukemia. *Am. J. M. Sc.*, 1944, **207**, 141–152.
16. Mallory, T. B., Gall, E. A., and Brickley, W. J. Chronic exposure to benzene (benzol). III. The pathologic results. *J. Indust. Hyg. & Toxicol.*, 1939, **21**, 355–393.
17. Rawson, R., Parker, F., Jr., and Jackson, H., Jr. Industrial solvents as possible etiological agents in myeloid metaplasia. *Science*, 1941, **93**, 541–542.
18. Martland, H. S. The occurrence of malignancy in radioactive persons; a general review of data gathered in the study of the radium dial painters, with special reference to the occurrence of osteogenic sarcoma and the inter-relationship of certain blood diseases. *Am. J. Cancer*, 1931, **15**, 2435–2516.
19. Firket, J., and Campos, E. S. Generalized megalokaryocytic reaction to saponin poisoning. *Bull. Johns Hopkins Hosp.*, 1922, **33**, 271–283.
20. Silberberg, M., and Silberberg, R. Action of estrogen on skeletal tissues of immature guinea pigs. *Arch. Path.*, 1939, **28**, 340–360.

21. Block, M., and Jacobson, L. O. Splenic puncture. *J. A. M. A.*, 1950, **142**, 641–647.
22. Wyatt, J. P., and Sommers, S. C. Chronic marrow failure, myelosclerosis and extramedullary hematopoiesis. *Blood*, 1950, **5**, 329–347.
23. Mossberg, H. Thrombocytopenia following local radiotherapy. *Acta radiol.*, 1947, Suppl. 67, 146 pp.
24. Myers, J. D. Personal communication.
25. Black-Schaffer, B., Johnson, D. S., and Gobbel, W. G., Jr. Experimental total midzonal hepatic necrosis. *Am. J. Path.*, 1950, **26**, 397–409.
26. Soskin, S., and Levine, R. Recent advances in physiology of the thyroid and their clinical application. *Arch. Int. Med.*, 1944, **74**, 375–383.

[*Illustrations follow*]

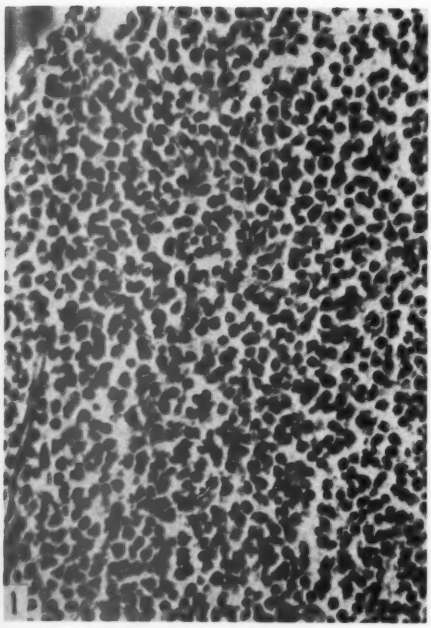
LEGENDS FOR FIGURES

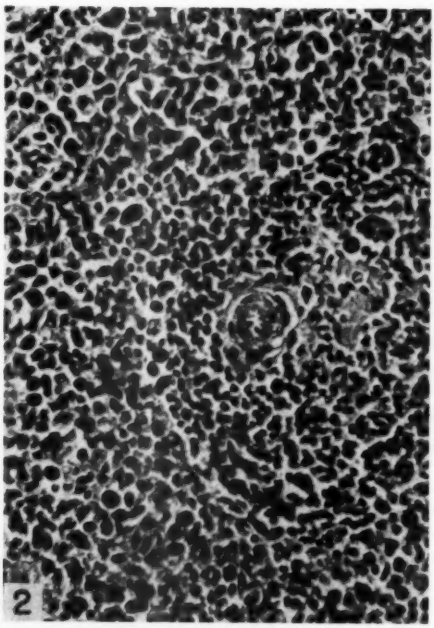
FIG. 1. Chronic granulocytic leukemia. Bone marrow. The cellular constituents are uniformly granulocytic. Cells with large round nuclei are myelocytes. Differentiated granulocytes have smaller lobulated nuclei. Case 5818. Hematoxylin and eosin stain. × 245.

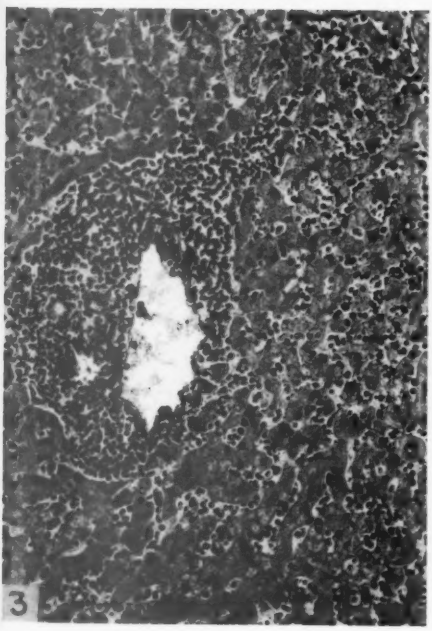
FIG. 2. Chronic granulocytic leukemia. Spleen. Granulocytic proliferation like that in the bone marrow obliterates the normal pattern of sinuses and cords on the left. The malpighian body at the right is composed chiefly of small lymphocytes, but larger myelocytes are beginning to replace them. Case 3538. Hematoxylin and eosin stain. × 245.

FIG. 3. Chronic granulocytic leukemia. Liver. The granulocytic proliferation conspicuously involves the portal area at the left. The cells in the sinuses also are of the granulocytic series. Case 5380. Hematoxylin and eosin stain. × 125.

FIG. 4. Chronic granulocytic leukemia. Lymph node. The capsule of the lymph node at the right and the adjacent cortex are sites of granulocytic proliferation like that in the bone marrow, the spleen, and the liver. At the bottom of the photograph are seen small lymphocytes remaining in the cortex. Case 5818. Hematoxylin and eosin stain. × 245.







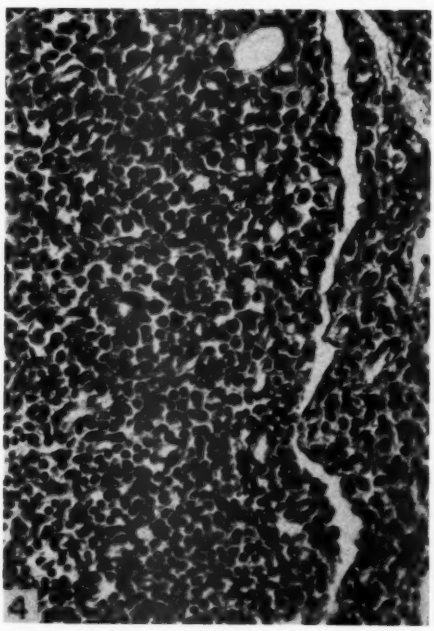
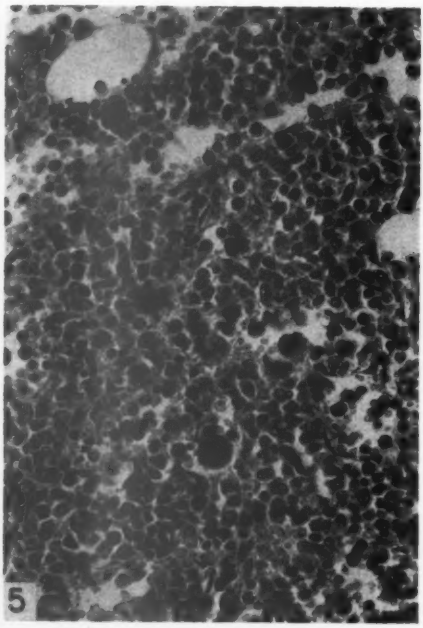


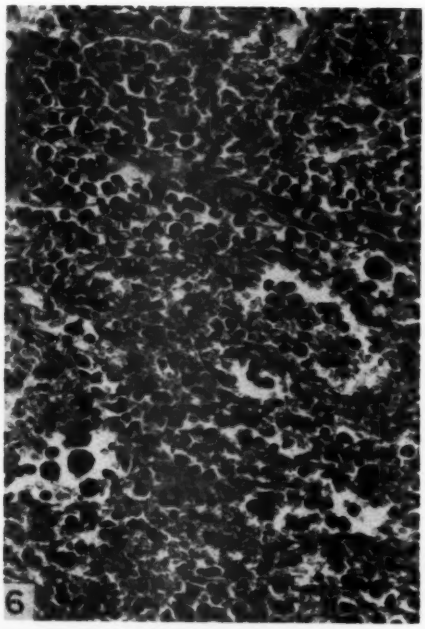
FIG. 5. Panmyelosis without myelofibrosis. Bone marrow. All normal hematopoietic cells are present in the hyperplastic marrow. Most of the cells are granulocytes; those with large, round reticular nuclei are myelocytes. A megakaryocyte lies in the center of the lower half of the field. In the upper left corner an island of erythropoiesis is marked by nucleated red blood cells that have small, round, uniformly dense nuclei. Case 2848. Hematoxylin and eosin stain. × 245.

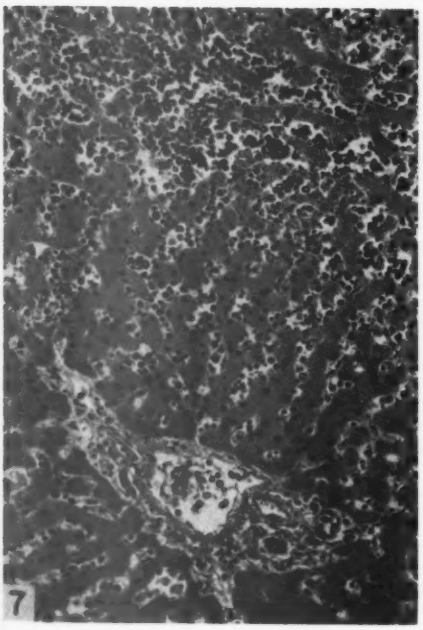
FIG. 6. Panmyelosis without myelofibrosis. Spleen. The architectural pattern of sinuses and cords remains. Within the lumina of sinuses and in the cords the hematopoietic proliferations are pleomorphic, as in the bone marrow. Case 2848. Hematoxylin and eosin stain. × 245.

FIG. 7. Panmyelosis without myelofibrosis. Liver. Unlike the portal areas in chronic granulocytic leukemia, that seen in the lower part of the field is not a site of hematopoiesis. Hematopoietic cells are present in the sinuses. Case 474. Hematoxylin and eosin stain. × 125.

FIG. 8. Panmyelosis without myelofibrosis. Lymph node. Pleomorphic hematopoietic cells are found in the cord jutting out from the right and in the surrounding sinus. The large cells are megakaryocytes. Granulocytes and a few nucleated red blood cells mingle with small lymphocytes. Case 2231. Hematoxylin and eosin stain. × 245.


5


6


7

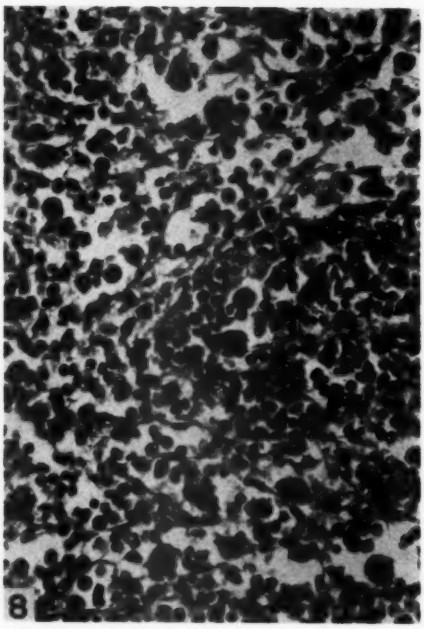
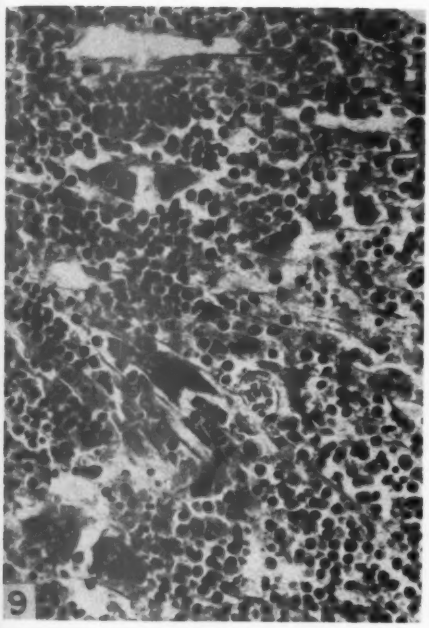

8

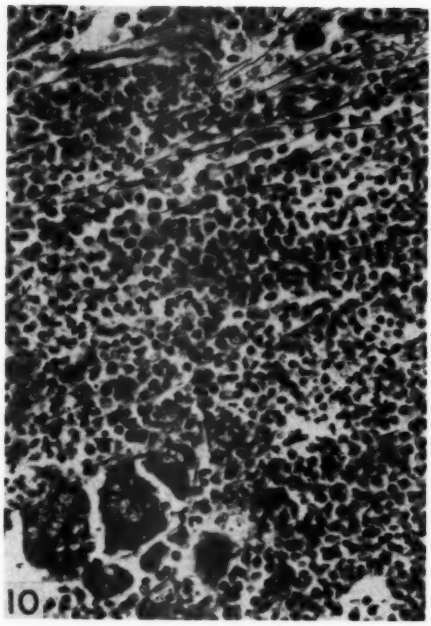
FIG. 9. Panmyelosis with myelofibrosis. Bone marrow. There is only minimal fibrosis in this case, and hematopoietic elements are hyperplastic. Megakaryocytes are conspicuous. In the upper left portion of the field are nucleated red blood cells containing round, uniformly dense nuclei. Most of the cells in the lower right are granulocytes. A mild increase in reticulin network is indicated by the fine fibers associated with small, spindle-shaped nuclei. Case 3125. Hematoxylin and eosin stain. × 245.

FIG. 10. Bone marrow from the same case as that illustrated in Figure 9, stained by Wilder's method for reticulin fibers. Fine, black argyrophilic fibers and associated reticulum cells with elongated nuclei are seen in the upper third of the field. Beginning fibrosis, as in this case, is not found uniformly throughout the marrow. × 245.

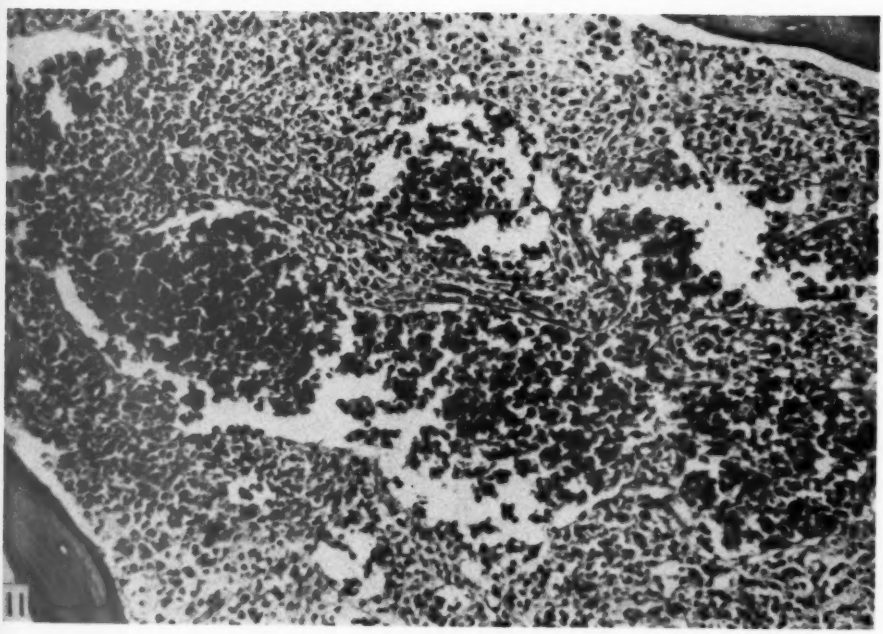
FIG. 11. Panmyelosis with myelofibrosis. Bone marrow. As fibrosis of the bone marrow increases, hematopoiesis occurs in greater degree within sinuses. In some foci, such as the area illustrated, the sinuses become widely dilated. Within these lacunar sinuses, hematopoietic cells are abundant and all normal types are represented. Case 4964. (See also Figures 12 and 13.) Hematoxylin and eosin stain. × 125.



9



10



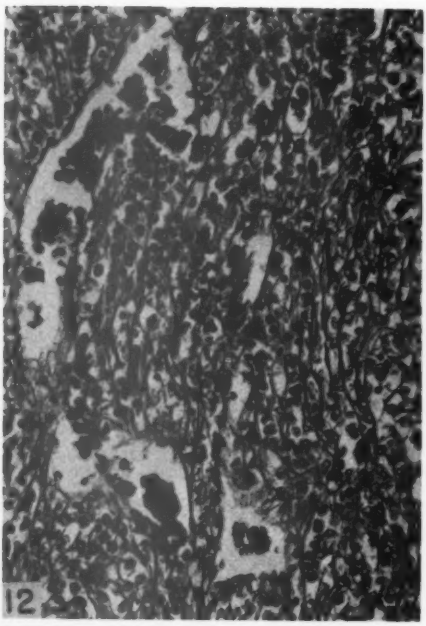
11

FIG. 12. Bone marrow from the same case as that illustrated in Figure 11, stained by Wilder's method for reticulin fibers. An abundant network of argyrophilic fibers appears amidst the hematopoietic tissue. A semilunar-shaped sinus at the left contains hematopoietic cells of various kinds. × 245.

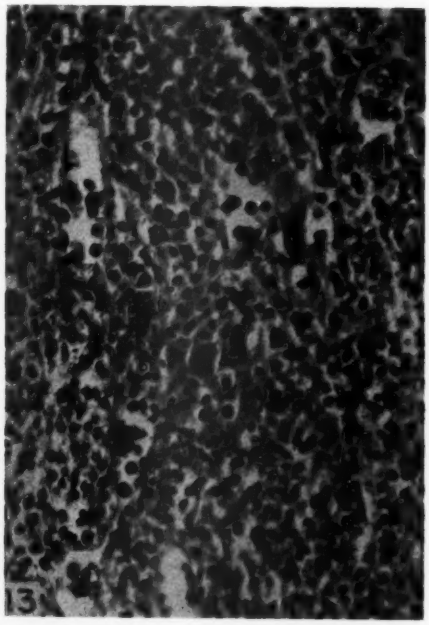
FIG. 13. The same case as that illustrated in Figure 12 and a similar area of the bone marrow stained with hematoxylin and eosin. Despite the abundance of fibers, many hematopoietic cells are present. To the left of center is an island of nucleated red blood cells. Two megakaryocytes are near the center. Most of the remaining cells are granulocytes, but the small elongated nuclei of cells associated with fibers also are seen. × 245.

FIG. 14. Panmyelosis with myelofibrosis. Bone marrow. When the bone marrow is altered by the extreme degree of fibrosis illustrated, few normal hematopoietic cells remain. Case 5335. Hematoxylin and eosin stain. × 245.

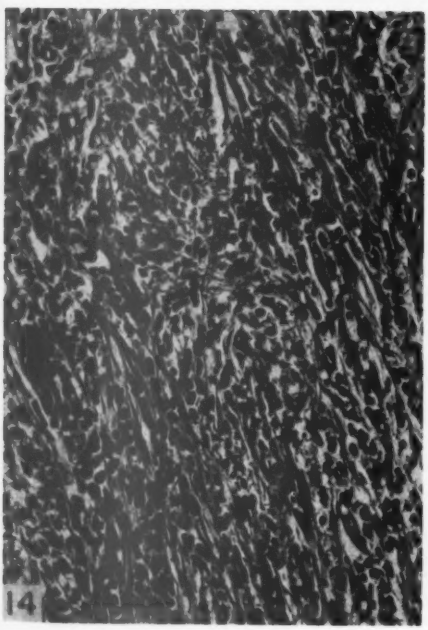
FIG. 15. A different area of the bone marrow from the same case as that illustrated in Figure 14. Little else but fibrous tissue occupies the spaces of the bone marrow, and the fibrosis is accompanied by new membranous bone formation, *i.e.*, osteosclerosis. On the right are periosteum and cortex; on the left, new, thick medullary trabeculae. New bone is identified by irregular contour of trabeculae, irregular lamellar pattern and calcification, and an increased concentration of osteocytes. Hematoxylin and eosin stain. × 64.



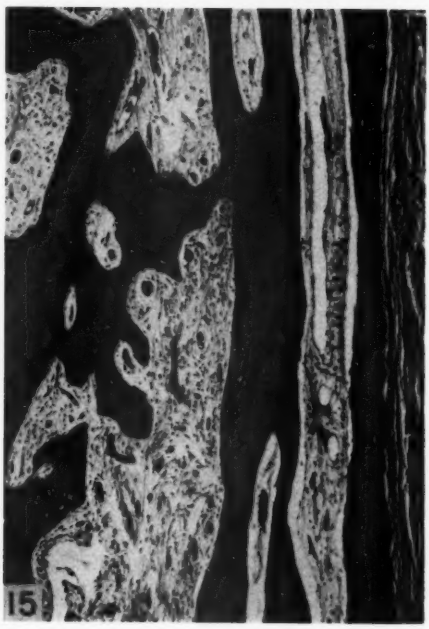
12



13



14



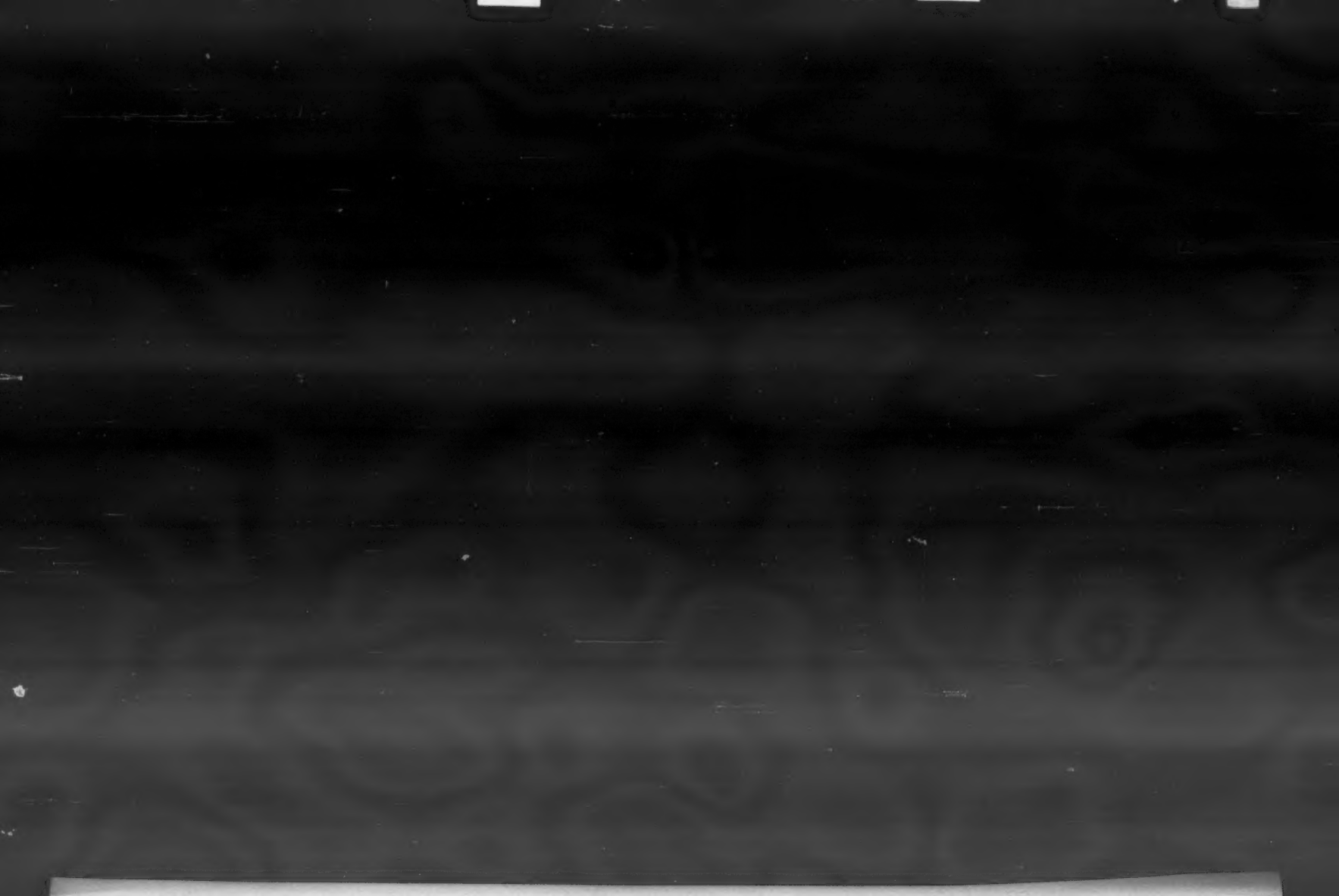
15

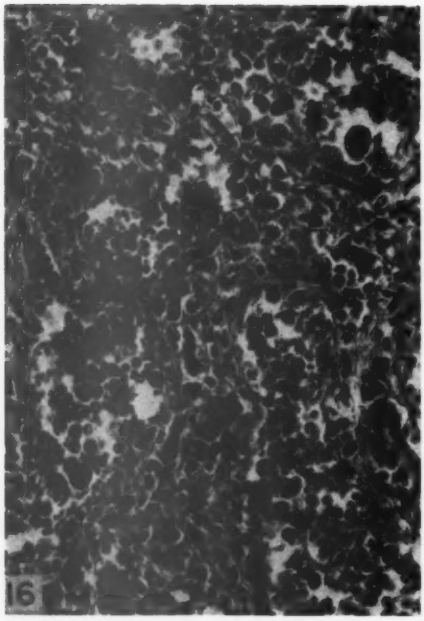
FIG. 16. Panmyelosis. Liver. In this case the liver is greatly altered, and many areas resemble bone marrow. The widely dilated sinuses contain hematopoietic cells of all kinds. The intervening hepatic parenchymal cells have become atrophic or have disappeared and have been replaced with blood-forming tissue lying in a delicate reticulum. Case 5335. Hematoxylin and eosin stain. × 245.

FIG. 17. Panmyelosis. Liver. In contrast with the liver illustrated in Figure 16, this liver is minimally altered. A few, large, multilobulated megakaryocytes and also granulocytes are present in the sinuses. Case 3125. Hematoxylin and eosin stain. × 245.

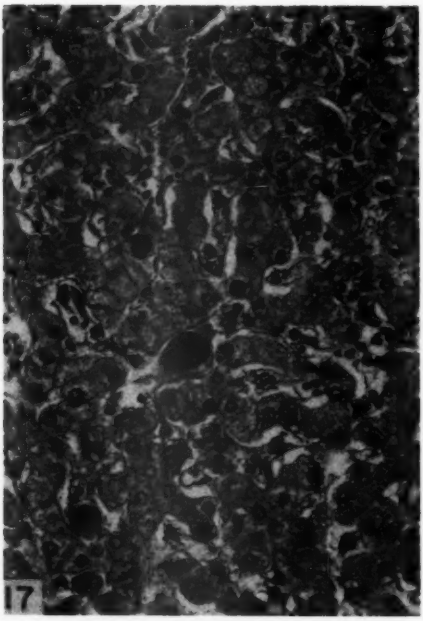
FIG. 18. Panmyelosis. An illustration of the degree of fibrosis occasionally found in lymph nodes. The normal architectural pattern is obscured by fibrosis. Megakaryocytes are seen in the lower half centrally. Granulocytes and nucleated red blood cells also are present amidst small lymphocytes. Case 5861. Hematoxylin and eosin stain. × 210.

FIG. 19. Panmyelosis. Kidney. The corticomedullary junctional area is illustrated. The interstitial tissue is infiltrated by hematopoietic cells, granulocytes for the most part. The degree of renal involvement in this case (5861) is unusual in panmyelosis. Hematoxylin and eosin stain. × 245.

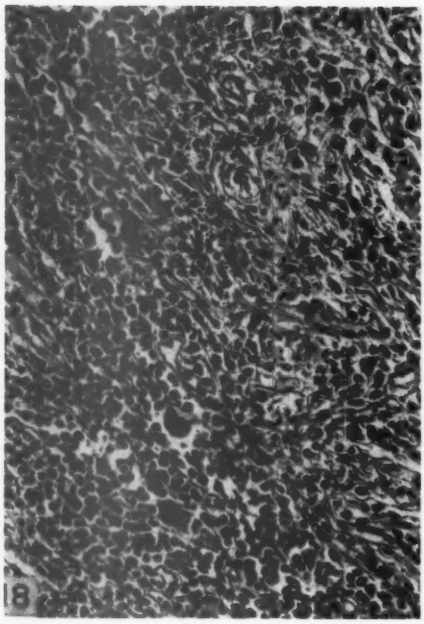




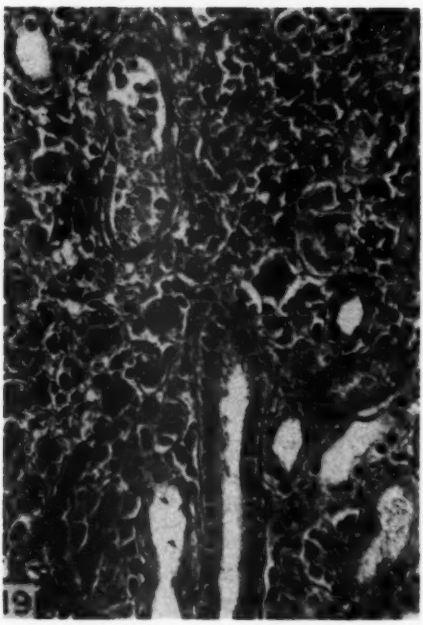
16



17



18



19

THE NATURAL HISTORY OF THE SARCOID GRANULOMA*

H. J. Barrie, B.M., and A. Bogoch, M.D.
(From the Department of Pathology, University of Toronto, Toronto, Ont.)

In the many papers on sarcoidosis, little attention has been paid to the earliest phase of development of the characteristic lesions of this disease. Most authors, like Pautrier[1] and Ricker and Clark,[2] believed that the aggregate of epithelioid cells is the earliest recognizable lesion. An earlier phase characterized by perivascular inflammatory reaction has been claimed by Kissmeyer,[3] described in the skin by Kyrle[4] and Lever and Freiman,[5] and in the heart by Johnson and Jason.[6] Little has been written about the significance of the granuloma in the natural history of sarcoidosis or the fundamental question whether the aggregates represent clumping of cells or the results of cell division. Furthermore, do the epithelioid cells represent a slow progressive reaction to some foreign body or are they sequelae of an acute exudative process?

To investigate these points, a study was made of the cases of sarcoidosis in the necropsy files of the Banting Institute. Several cases were rejected because the lesions were open to question or because the picture was confused by coincident disease such as disseminated lupus and widespread deposits of a seminoma. There remained 24 cases suitable for this study. A full description of the distribution of the lesions in this series of cases is being published separately. Use was made also of biopsy material obtained through Dr. W. L. Robinson from the Department of Surgical Pathology.

Age Changes in the Granuloma of Sarcoidosis

It was easier to find changes indicative of increasing age in the granulomata than to determine the duration of the disease in any one case; therefore, the life cycle of the granuloma as a histologic structure will first be described.

Mature Granulomatous Lesion

The characteristic lesion of sarcoidosis has been described so often that we need mention only those features which relate to its genesis. The lesions are roughly spherical and the plump or fusiform epithelioid cells are often cupped into one another as if by pressure. The granuloma contains reticulin fibers and it often has been assumed that these represent "inclusions" of the original stromal fibers of the tissue

* Presented at the Forty-ninth Annual Meeting of the American Association of Pathologists and Bacteriologists, New York City, April 10, 1952.
Received for publication, October 23, 1952.

involved. We believe, however, that these fibers are produced by the epithelioid cells at an early stage of development because "inclusions" of collagen fibers are absent and reticulin may be found readily around new-formed epithelioid cells. Furthermore, the very early lesions may contain no reticulin at all (Fig. 1). The original stroma of the tissue involved is displaced to the periphery of the granuloma as an even, concentric, compression capsule, which later becomes thickened and hyaline when eccentric peripheral fibrosis occurs in the retrogressive stage of the lesion. A mature sarcoid lesion may be considered wrongly to contain collagen fibers if it is not appreciated that a large aggregate is commonly composed of two to four semi-confluent miliary granulomata which have formed along the course of a small vessel. The resultant structure resembles a shrub with several nests of sarcoid granuloma in its branches as shown in Figures 1 and 2, in which one miliary granuloma has taken shape and others are developing. When a band of collagen appears to be in a central position, it is actually part of the capsules of aggregated miliary granulomata.

The cupping of the cells and the formation of a compression capsule are strongly in favor of the granuloma being formed locally, not by aggregation of cells, but by cell proliferation. However, though the fully formed epithelioid cells sometimes stain intensely with eosin and hematoxylin, we have never seen indisputable mitotic figures in them and it is doubtful if they are capable of further complete division. Amitotic nuclear division in a mature epithelioid cell seems to result in the formation of multinucleated cells. Therefore the aggregate must be formed initially by division of a parent cell of the epithelioid cell or of an intermediate form.

The Early Phase

In the study of the early phase of sarcoidosis it soon became apparent that there was evidence to support the belief that the granuloma develops by cell proliferation. Immature granulomata were common in the cases listed in Table I and had the following characteristics:

Cell Pleomorphism and Hyperchromatism. Besides a few fully formed epithelioid cells with their pale nuclei, there were numerous smaller, usually fusiform cells with more angular, darkly staining nuclei and relatively scanty, angulated eosinophilic cytoplasm (Fig. 2); and accompanying them were abundant mononuclear cells and transitional forms. These young cells had the appearance one associates with cells in active division, but the mitotic figures were rare and

only occasionally seen in the transitional forms. It was of course difficult to identify from a "still" picture the exact parent cell of the epithelioid cell and we have no means of determining if lymphocytes as well as monocytes played an important rôle. Multinucleate cells were already forming and had not the abundance of nuclei found later but contained two or three darkly staining nuclei (Fig. 3).

Disorder in Cellular Arrangement. The early fusiform hyperchromatic cells were not cupped into one other but were grouped in a haphazard manner or were arranged in bands of parallel cells resembling a sarcoma. These ill defined cellular masses lacked a compression capsule but the latter was beginning to form around small aggregates because of local hypertrophy of the epithelioid cells (Figs. 1, 2, and 3).

The Presence of a Mononuclear Cell Infiltration in a Wider Area Than the Field of the Granuloma. Mononuclear cell infiltration in a wider area than the field of the granuloma was most striking in the lungs (Figs. 4 and 5) where the early granuloma, sometimes interstitial but often intra-alveolar, was surrounded by a nebula of lymphocytes and monocytes, gradually fading out into the surrounding parenchyma. The less mature the granulomata, the wider the cellular infiltration; and, conversely, once the sarcoid lesions had become mature, the cloud of infiltration had largely disappeared. This mononuclear cell infiltration, usually accompanied by destruction of parenchyma, was also found in the liver (Fig. 6), parotid gland (Fig. 7), heart (Fig. 8), and central nervous system. This feature was so marked that it is strange so few observers have mentioned it. As will be indicated in the paragraph on retrogressive lesions, this diffuse inflammation leaves behind it a variable amount of fibrosis. While granulomata in a proliferative phase were found in the spleen, nodes, and bone marrow, the mononuclear cell infiltration in these organs was either absent or difficult to recognize.

Sarcoidosis, as it affects certain organs, appears to progress through three stages: (1) diffuse inflammation; (2) cell proliferation leading to the formation of epithelioid cells; (3) hypertrophy of epithelioid cells to form localized cell aggregates. We have not made a special study of sarcoidosis in the skin but from the papers of Kyrle[4] and Lever and Freiman[5] we may judge that a similar process occurs there.

Retrogressive Lesions

The sarcoid granuloma is an avascular structure dependent for its life on the diffusion of fluids from nearby vessels. If, as we believe, the granuloma is a response to some foreign agent, the stability or

otherwise of the granuloma may throw light on the nature of the causative agent, besides reflecting the influences of natural ageing and of changes in the surrounding circulation.

The histologic pattern in a group of nodes may appear astonishingly stable, as has been shown by biopsy after a long interval from the site of a previous excision. The tendency for sarcoidosis to persist apparently unaltered for long periods is also evident clinically in some cases. This does not enable us to assert that the foreign agent has no deleterious effects on its host, the epithelioid cell, for death of an epithelioid cell might be followed by local replacement, and most granulomata contain occasional mononuclear cells from which such replacement could be derived.

Retrogressive changes may be divided into active degeneration, simple atrophy, and fibrosis.

Active Degeneration. Many forms of active degeneration other than simple atrophy occurred in the cells composing the granulomata. Thus lysis, fragmentation, and coagulative necrosis were encountered frequently. Lysis was most apparent in the nuclei, especially those of giant cells which were often reduced to shadowy outlines (Fig. 9). This aroused interesting speculation regarding the field of cytoplasm related to these nuclei. It is possible that some of the calcified Schaumann bodies might have had their origin in micro-infarcts. Fragmentation of the cytoplasm was common, although it was always difficult to exclude the possibility of artefact. Coagulative necrosis occurred in the centers of the granulomata and has often been described. There is no justification for calling it fibrinoid necrosis. It has a slightly refringent quality and is more eosinophilic than the caseous necrosis in tuberculosis.

In the majority of instances, when active degenerative changes were occurring, the cells in the immediate neighborhood showed signs of increased activity, made evident by irregularity of outline, hyperchromatism and irregularity of the nucleus, and the presence of small multinucleated cells containing only two or three nuclei (Fig. 10).

Simple Atrophy. Many of the cases showed simple atrophy of the granuloma. Here the epithelioid cells showed indistinct outlines. The chromatin stained poorly and was limited to the periphery of the nuclei. They were surrounded by little clusters of lymphocytes which were sharply limited to the lymphatic pathways in contrast to the diffuse exudate found in the early stage (Fig. 11). The multinucleated cells often survived the longest, as was also the case when the lesion underwent fibrosis.

The difference between the slow atrophy and the more active degen-

erative lesions apparently was not related to disturbances in the surrounding vascular system and we believed it more probably represented differences in the degree of activity of the causative agent.

Fibrosis. There is ample clinical evidence that lesions in the eye and skin, and therefore probably elsewhere, may undergo atrophy and absorption without fibrosis. In 7 of the cases, however, the latter was a marked feature. This process may also provide clues to the significance of the granuloma.

Fibrosis occurred in two forms. In one, the fibrous tissue was arranged in parallel strands and its relation to granuloma formation was not always obvious. It is probable that some of it represented a sequel of the diffuse mononuclear cell infiltration we have already described. In the other form, fibrosis was nummular and obviously related to the granulomata. It always started in the periphery of the granuloma though it was often eccentric and, because of the compound nature of the aggregates, often partially divided a granuloma into the shape of a heart. It was usually hyaline and in 3 cases there was material present which showed the staining reaction of amyloid. It usually has been assumed that the epithelioid cells become transformed into fibroblasts but the evidence for this is very scanty. Hortega's silver carbonate stain encrusts fibroblasts well but not epithelioid cells. Using this technique it was seen that the onset of the fibrosis was associated with the migration of fibroblasts into the periphery of the granuloma. This occurred only when the epithelioid cells were disappearing. As long as epithelioid cells remained, all extracellular fibrils in the granuloma were slender and argyrophilic and could be classed as reticulin. As the epithelioid cells disappeared, two processes led to fibrosis. One was the migration of fibroblasts into the periphery and the other was the gradual coarsening of the reticulin fibers of the granuloma into hyaline collagen. There was no evidence to suggest that the epithelioid cell ever took on the function of a fibroblast. We take the opposite standpoint and assert that the granuloma, while viable, is remarkably resistant to fibrosis. The tendency for the giant cells to persist longest in a hyaline lesion is illustrated in Figure 12.

We may remark here, too, that besides being resistant to fibrosis, the small complex of epithelioid cells and reticulin fibers was found to be relatively impenetrable to polymorphonuclear infiltration and, in pneumonia, could be found like a little island in a sea of polymorphonuclear cells. The little granuloma has therefore a great capacity for isolation and this may be the clue to its function, as discussed in a later paragraph.

In some of the cases showing the most fibrosis, doubly refractile

particles were present in the granuloma. That some of this was silicon dioxide was made likely by the presence of silicotic lesions elsewhere. The epithelioid cells and giant cells often contained carbon particles also and it is obvious that their load may not be confined to any one foreign substance. Coincident phagocytosis of silicon dioxide would be expected to modify the natural history of the sarcoid lesion.

Age of the Disease

So far, we have been dealing with histologic features only. To what extent does our division of the lesions of sarcoidosis into early, mature, and retrogressive types agree with the clinical course of the disease? A major difficulty here was the common absence of symptoms referable to sarcoidosis and in 18 of our cases the disease was an accidental finding, death being due to other causes. Even when death was due to pulmonary insufficiency as in case 12 there was no means of determining how long sarcoidosis had been present in the lungs before symptoms occurred. A further but lesser difficulty lay in the fact that in many cases the various organs contained lesions of different ages. This corresponds to the well known clinical experience that sarcoidosis has recurrent phases of activity. Nevertheless, by taking into account the age of the majority of the lesions, it was possible to divide the cases into those in which the lesions were predominantly early, mature, or retrogressive, respectively, and the resulting correlations with the clinical findings are shown in Tables I, II, and III. It can be seen that all 4 cases with cardiac involvement fall into the early group, as sarcoidosis of the heart shows all the features which we have described as the early phase.

That this probably did represent an early phase and was not merely due to some special reaction of the myocardium was shown by the fact that in 3 of the 4 cases with involvement of the heart, two other organs also contained early lesions. In the group with retrogressive lesions, there were no scars in the heart to suggest that sarcoidosis had been present at one time and had healed. The average age for the cases in Table I was 43, in Table II, 48, and in Table III, 60 years. The significance of this would be hard to define on statistical grounds. Clinical data can be seen to be of little help except in case 4 which has been published by Oille, Ritchie, and Barrie[7] and must be mentioned again here. This was the case of a 43-year-old woman who, because of symptoms of an iron-deficiency anemia, had an electrocardiogram and fluoroscopic examination of the lungs in May, 1948. Both were normal. In August she was febrile for a week and suffered pain in the

TABLE I
Cases Showing Mainly Early Lesions

Case no.	Sex	Age	Organs involved	Early		Mature	Retrogressive			Cause of death	Symptoms of sarcoidosis	Associated disease
				Diffuse inflammation	Proliferation		Active degeneration	Atrophy	Fibrosis			
1	F	51	Heart Liver	+	+	 +			+	Heart failure at operation	None	Goiter
2	M	35	Lung Nodes Hypothalamus Spleen	+	+ + +	+++ +	+			Hypothalamic sarcoid lesion	Pain in legs for 10 months, weakness for 3 months (diabetes insipidus for 6 years)	
3	F	14	Lung Spleen Nodes Liver	+ +	+ + + +	+ + + +				Operative shock	None	Congenital heart disease, rheumatic myocarditis and endocarditis
4	F	43	Lungs Heart Liver Nodes Pituitary body	+ + +	+ + +	 + +				Cardiac sarcoid	Acute illness for 3 months, fatigue for 4 weeks	Iron deficiency anemia
5	F	71	Liver Nodes		+	 +	 +		 Early	Heart failure		Hypertension, silicosis (mild)
6	M	36	Heart Liver Lungs Nodes Spleen	+ +	+	 + + +			 +	Bronchopneumonia and abscess of lung aggravated by sarcoidosis	Uncertain	Asthma, recurrent; pneumonia since childhood
7	M	50	Lung Spleen Node	+(slight)	+ ?	 + ++	 + +		 + +	Meningococcal septicemia	None	Chronic bronchitis, alcoholism
8	F	48	Liver Lung Nodes Pancreas Kidney Heart Spleen Appendix	 + +	 +++ + +	 + + + +		+ + +		Cardiac sarcoid	4½ months	Pernicious anemia

TABLE II

Cases Showing Mainly Mature Lesions

Case no.	Sex	Age	Organs involved	Early		Mature	Retrogressive			Cause of death	Symptoms of sarcoidosis	Associated disease
				Diffuse inflammation	Proliferation		Active degeneration	Atrophy	Fibrosis			
9	M	46	Spleen Lungs Liver			+ + +				Bronchopneumonia	None	Meningioma
10	F	21	Lungs			+				Subarachnoid bleeding	None	
11	M	59	Nodes Lungs Kidneys Liver		 +? 	 + + +			+ + (early) + (early)	Uremia	None	Hyperplasia of prostate
12	F	66	Spleen Lung			+			 +	Fat embolism	None	Fracture of mandible
13	F	43	Kidney Skin Lungs Spleen Appendix Marrow		 +?	+ + +++ + + +	 + 		+ (early) ++ (early)	Pulmonary insufficiency	Cough for 3½ years, lesions in lungs by x-ray for 2 years, nodal enlargement for 16 months	
14	M	49	Node Liver	 +		+ +				Cerebral hemorrhage	None	Hypertension
15	M	54	Nodes Spleen Lungs Liver		+	+ + + +	+	 + 	+ (early)	Rupture of esophageal varix	None	Cirrhosis, diabetes

TABLE III

Cases Showing Mainly Retrogressive Lesions

Case no.	Sex	Age	Organs involved	Early		Mature	Retrogressive			Cause of death	Symptoms of sarcoidosis	Associated disease
				Diffuse inflammation	Proliferation		Active degeneration	Atrophy	Fibrosis			
16	F	76	Node Liver Kidney Lungs			+ + + +		 ++	 +	Operative shock	None	Fracture of femur
17	M	62	Lungs					+		Cardiac infarct	None	
18	M	63	Lungs Nodes					+	 +	Cardiac infarct	None	Diabetes, cirrhosis
19	M	64	Lungs Spleen Nodes					+	 + +	Bronchopneumonia aggravated by sarcoidosis	None	Carcinoma of colon, bronchiectasis
20	M	51	Lungs Spleen Liver Nodes			+		+	 + ++ ++	Bronchopneumonia	None	Carcinoma of tongue
21	M	71	Lungs Spleen Nodes					+	 + +	Bronchopneumonia	None	Carcinoma of esophagus
22	M	40	Liver Spleen		 ?	+ +	+		+(early)	Cerebral hemorrhage	None	Hypertension
23	M	58	Lungs Pericardium Liver Nodes Spleen			+ + + +		+	 + +(early) ++ +	Fracture of skull	None	Hypertension
24	M	58	Lungs Nodes		?			+	 +++	Bronchopneumonia	None	Mild silicosis

ears and swelling of the lymph nodes. She then developed a slight cough. An electrocardiogram was again normal on October 26 but 2 weeks later she suddenly became so tired that she could not get out of bed. Two weeks after this she was unexpectedly found dead in bed. At necropsy, the heart was widely infiltrated by sarcoidosis and there were also lesions in the liver, lungs, and lymph nodes. Invasion of the heart may be assumed to have occurred after the last normal electrocardiogram and the cardiac lesions, therefore, were less than 4 weeks old. This remarkable case gives us a time standard with which to judge the development of the cardiac lesions of sarcoidosis, and the patient's febrile episode 3 months before death strongly suggests that that was the time of onset of the diffuse inflammatory phase.

We had no clues from our material which would reveal how long the mature and retrogressive lesions had been present in the body. Case 2 was difficult to interpret. Proliferative lesions were present in lungs, spleen, and hypothalamus in spite of the fact that it was probable that diabetes insipidus of 6 years' standing was due to sarcoidosis of the mid-brain.

The tables emphasize the benign nature of the disease. Death was caused by sarcoidosis in only 6 cases and in 5 of these there was involvement of either the heart or the central nervous system.

Discussion

It would seem that certain deductions can reasonably be made from a study of the natural history of sarcoidosis as it has been outlined and from the facts already available in the literature. For purposes of comparison, a brief survey of granulomas of some other types might be helpful.

The granulomatous reaction is a response to a foreign agent either introduced from without or produced by modification of some normal structure in the body. Exogenous agents may be divided into those which are not capable of self-propagation and those which are. Of the first, the best example is silicon dioxide. This slowly soluble but highly active substance has a portal of entry (the lungs), and the granulomatous response is found along the migratory routes of the monocytes which phagocytize the particles. A certain concentration of the agent is necessary for granuloma formation and therefore the lungs and the sites nearest to them contain the more numerous granulomata. It is probable that individual monocytes containing silicon dioxide may be present anywhere in the body for they can migrate through the walls of pulmonary veins and enter the blood stream, but no granuloma

develops because of the small amount of silica present. In the liver, spleen, and marrow, where blood-borne silica is filtered out and concentrated, it is well known that silicotic nodules may develop.

Of exogenous agents capable of proliferation, an important example is *Mycobacterium tuberculosis*. With this agent the localization of lesions is not controlled by the initial concentration alone, but also by the ability of the migrating bacterium to proliferate in its immediate surroundings. The portal of entry in the majority of cases is the same as in silicosis. Thus tubercles develop at the same sites as silicotic nodules as well as in certain organs which seem to favor the proliferation of tubercle bacteria, such as the genito-urinary tract and the central nervous system.

Finally, granulomas may develop from a modification of some normal component of the body. Sometimes this is a localized phenomenon such as the formation of giant cells around altered elastica in a locally destructive lesion in the lung, but here the cause is usually obvious. When generalized and due to abnormalities of antigen-antibody reaction, the primary lesion occurs in components of the vascular system rather than in individual organs and the distribution of lesions is very wide, including skin, serous surfaces, joints, muscles, and viscera. Sometimes, however, diseases believed to be due to hypersensitivity, such as rheumatic fever, may show a remarkable organ specificity which may affect the heart alone; arsenical reactions may affect the heart and skin, and "malignant granuloma" affects the midline of the face.

Against this background the main features of the lesions in sarcoidosis may be reviewed. On such limited material, no dogmatic statement is possible about the significance of the diffuse mononuclear cell infiltration that was found in the lungs, liver, heart, salivary glands, and central nervous system, and which probably occurs also in the skin. It remains, however, an interesting finding and opens the possibility that it represents one of the missing links in sarcoidosis—the stage of active organ invasion.

The first stage that we can definitely recognize as being part of the disease is an early proliferative phase which is recognizable in all sites in which the granuloma may be found, that is, in the lymphatics, in the migratory routes of the phagocytes in the organs mentioned, and in the filter systems of the body, the lymph nodes, the spleen, and the marrow. A reasonable interpretation of this sequence is that some foreign agent is present that is capable of damaging parenchymal cells and producing some fibrosis. This foreign agent is removed from contact

with the tissues by phagocytosis. The phagocytes, however, may themselves be damaged by the (foreign) agent, react by proliferation and then by hypertrophy, and become transformed into epithelioid cells.

Proliferation of phagocytes and enlargement of the daughter cells continue to a point at which some stability occurs. It would seem most reasonable to suggest that this point of stability is reached when the foreign agent is diluted sufficiently by successive division of epithelioid cells to impair its capacity to induce further cell division. The granuloma thus represents merely a stabilization of the phagocytic process, the purpose of which is to isolate the foreign agent. That the latter may finally be dissolved, neutralized, or removed is suggested by the frequent disappearance of the granuloma. That it may damage the epithelioid cells themselves has been considered in the discussion on retrogressive changes.

When the epithelioid cell dies as a result of this injury three things may occur: Firstly, if the epithelioid cell is placed centrally it may remain *in situ* and give the picture of coagulative necrosis. Secondly, if placed peripherally, the epithelioid cell may be absorbed. The foreign agent may then be liberated and stimulate the peripheral fibrosis that is common in old sarcoid lesions. Thirdly, the liberated foreign agent may be picked up once more by phagocytes and the granuloma thus may extend and may coalesce with other lesions.

When the behavior of sarcoid is compared to the three types of granuloma that have been discussed, it can be seen at once that it is more complicated than the behavior of the body to silica. The distribution of the earliest lesions in lung, liver, skin, central nervous system, and salivary glands makes it tempting to suggest a virus as the etiologic agent. Search for a virus in sarcoidosis has so far been unproductive but it is possible that these observations have been made at an unsuitable phase in the development of the lesion. By the time the granulomas of sarcoid have become established they may not be harboring an active virus but only some changed component of the virus or of the body's tissues.

In the precise differentiation between the rôle of hypersensitivity and organismal invasion the threads of the clues run out, but it is believed that the interpretation of the disease up to this point may have value in understanding its natural history.

Summary

Formation of the sarcoid granuloma is often preceded by a phase of diffuse mononuclear cell exudation.

The granuloma is formed first by proliferation and then by hypertrophy of monocytes.

Early sarcoid lesions may therefore be recognized by three features: pleomorphism and hyperchromatism of the cells; disorder in cellular arrangement; the presence of an outlying infiltration of mononuclear cells.

Of 24 necropsied cases of sarcoidosis, the predominant lesions were early in 8, mature in 7, and retrogressive in 9.

The granuloma does not undergo fibrosis by a change of the epithelioid cells into fibroblasts. Fibrosis occurs from the periphery and follows atrophy or necrosis of the epithelioid cells.

The granuloma is probably a protective device whose function is to isolate some substance from the body tissues. Proliferation of monocytes and hypertrophy into epithelioid cells may dilute this substance to a point where it has little further action on the phagocytes. The granuloma then becomes relatively stable.

Breakdown of the epithelioid cells may liberate this substance, which then causes fibrosis or is re-phagocytosed.

REFERENCES

1. Pautrier, L. M. La maladie de Besnier-Boeck-Schaumann. Masson et Cie, Paris, 1940, 344 pp.
2. Ricker, W., and Clark, M. Sarcoidosis. A clinicopathologic review of three hundred cases, including twenty-two autopsies. *Am. J. Clin. Path.*, 1949, **19**, 725–749.
3. Kissmeyer, A. La maladie de Boeck: Sarcoïdes cutanées bénignes multiples. Levin & Munksgaard, Copenhagen, 1932, 147 pp.
4. Kyrle, J. Die Anfangsstadien des Boeckschen Lupoids; Beitrag zur Frage der tuberkulösen Ätiologie dieser Dermatose. *Arch. f. Dermat. u. Syph.*, 1921, 131, 33–68.
5. Lever, W. F., and Freiman, D. G. Sarcoidosis. Report of an atypical case with erythrodermic lesions, subcutaneous nodes and asteroid inclusion bodies in giant cells. *Arch. Dermat. & Syph.*, 1948, **57**, 639–654.
6. Johnson, J. B., and Jason, R.S. Sarcoidosis of the heart. Report of a case and review of the literature. *Am. Heart J.*, 1944, **27**, 246–258.
7. Oille, W. A., Ritchie, R. C., and Barrie, H. J. The age of the lesions in a case of cardiac sarcoidosis. *Canad. M. A. J.*, 1953, **68**, 277–278.

[*Illustrations follow*]

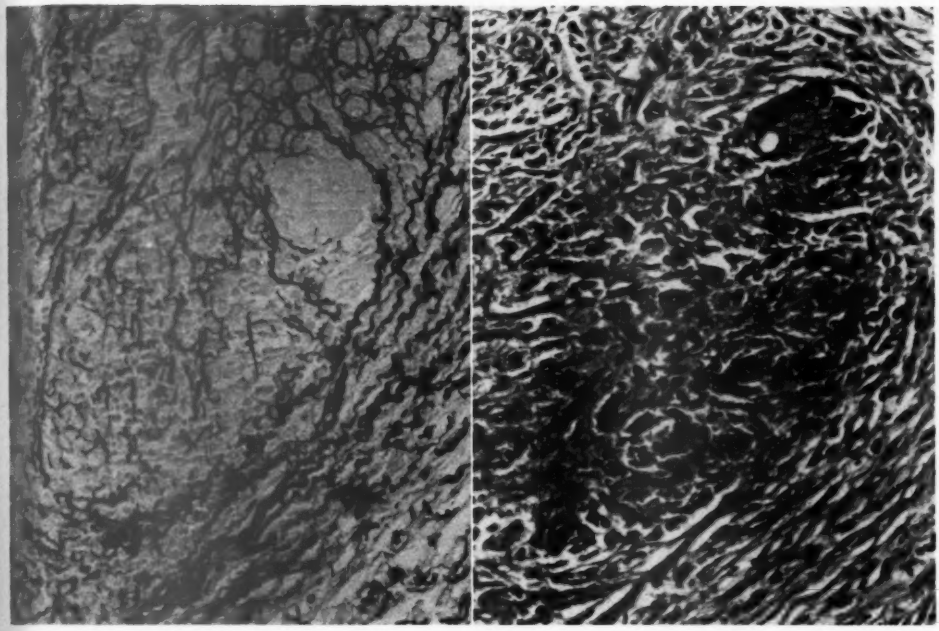
LEGENDS FOR FIGURES

FIG. 1. Early sarcoid nodule. One miliary granuloma is already formed but as yet contains no reticulin. Surgical specimen for biopsy of retro-orbital tumor. Laidlaw's stain. × 256.

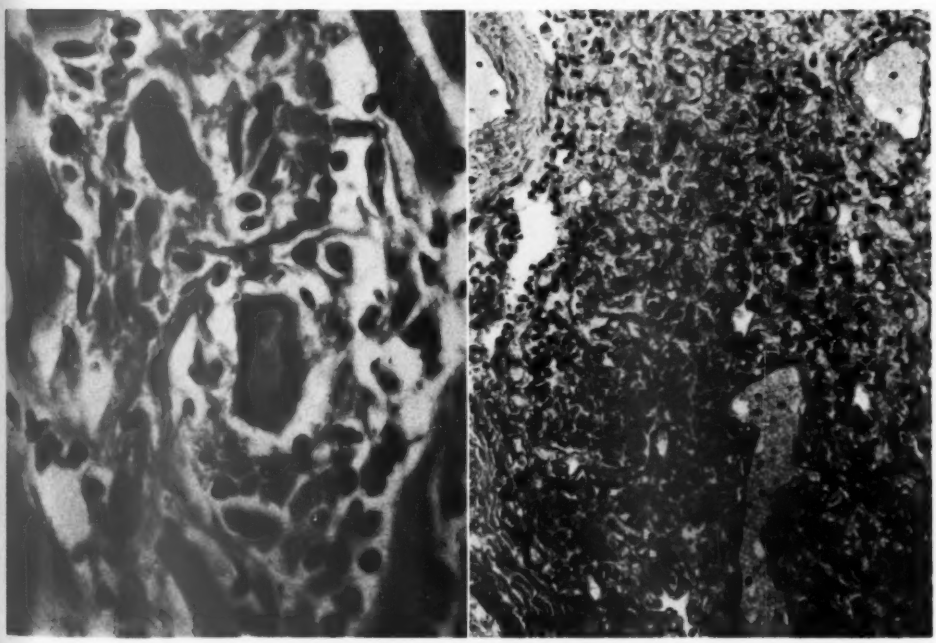
FIG. 2. Stage of cell division in early sarcoid. Same section as Figure 1. Hematoxylin and eosin stain. × 256.

FIG. 3. Case 6. Early sarcoid granuloma in heart. Stage of cell division. Hematoxylin and eosin stain. × 538.

FIG. 4. Case 3. Diffuse mononuclear cell exudate surrounding an early granuloma in the lungs. Hematoxylin and eosin stain. × 128.



1 2



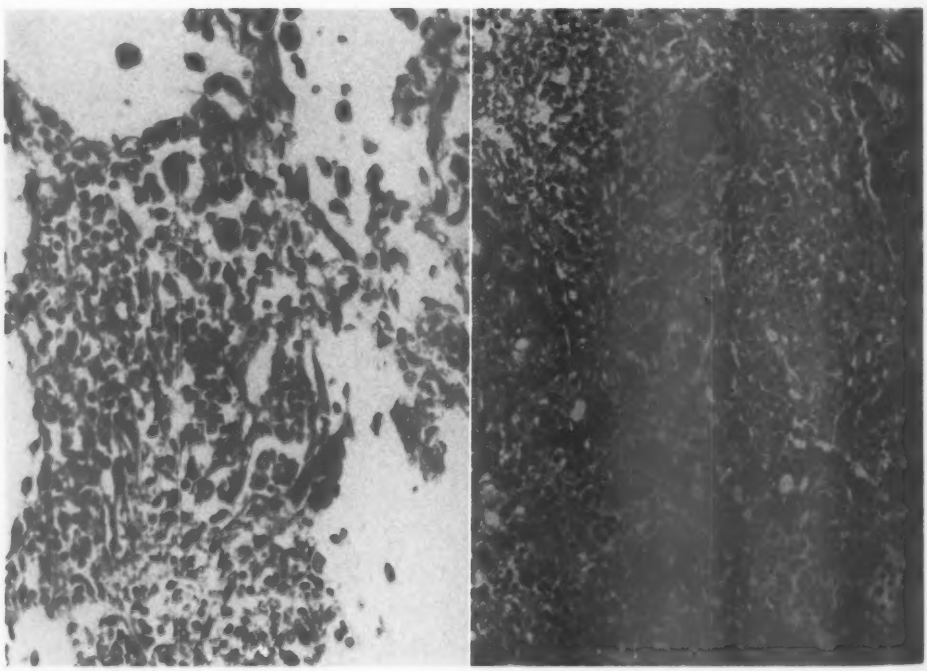
3 4

FIG. 5. Case 4. Diffuse mononuclear cell exudate and very early sarcoid aggregate in lungs. Hematoxylin and eosin stain. × 256.

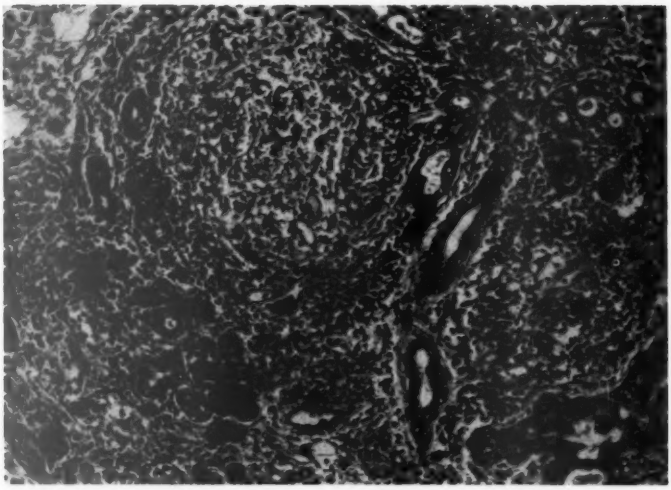
FIG. 6. Case 4. Diffuse mononuclear cell exudate and loss of parenchyma in the liver. A sarcoid giant cell is present in the upper portion of the field. Hematoxylin and eosin stain. × 128.

FIG. 7. Diffuse mononuclear cell exudate in parotid gland. An early sarcoid granuloma is visible in the upper portion of the field. Surgical biopsy. Hematoxylin and eosin stain. × 128.

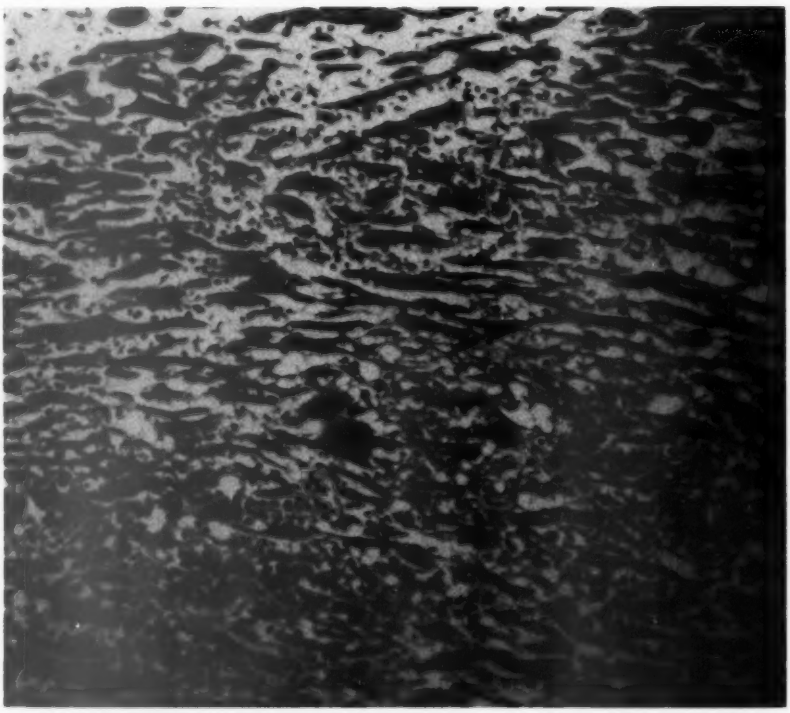
FIG. 8. Case 4. Diffuse mononuclear cell infiltration and atrophy of fibers at periphery of sarcoid granuloma in heart. Hematoxylin and eosin stain. × 54.



5 6



7



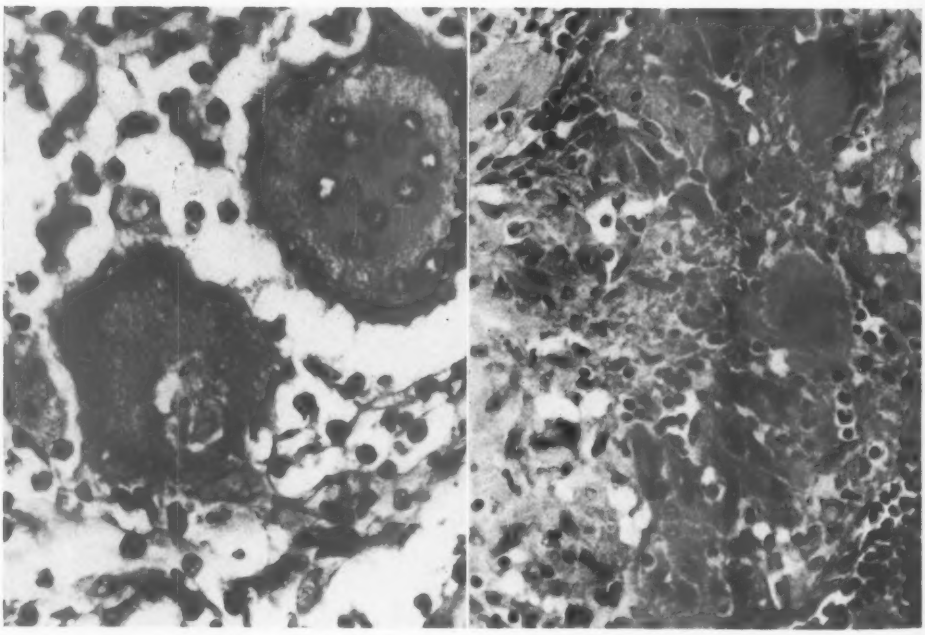
8

FIG. 9. Case 4. Lysis of nuclei in one giant cell and a Schaumann body in the other. Hematoxylin and eosin stain. × 538.

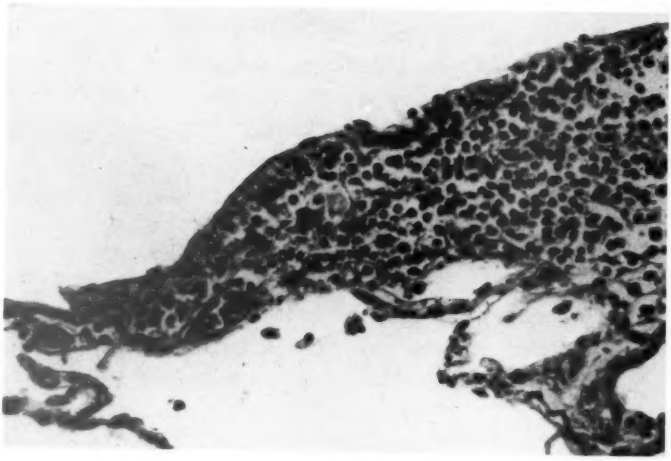
FIG. 10. Coagulative necrosis in the center of a sarcoid aggregate. The neighboring epithelioid cells show signs of activity. Surgical specimen of lymph node. Hematoxylin and eosin stain. × 256.

FIG. 11. Case 16. Atrophic sarcoid lesion. A few residual epithelioid cells are accompanied by lymphocytes sharply limited to the lymphatic pathways. Hematoxylin and eosin stain. × 256.

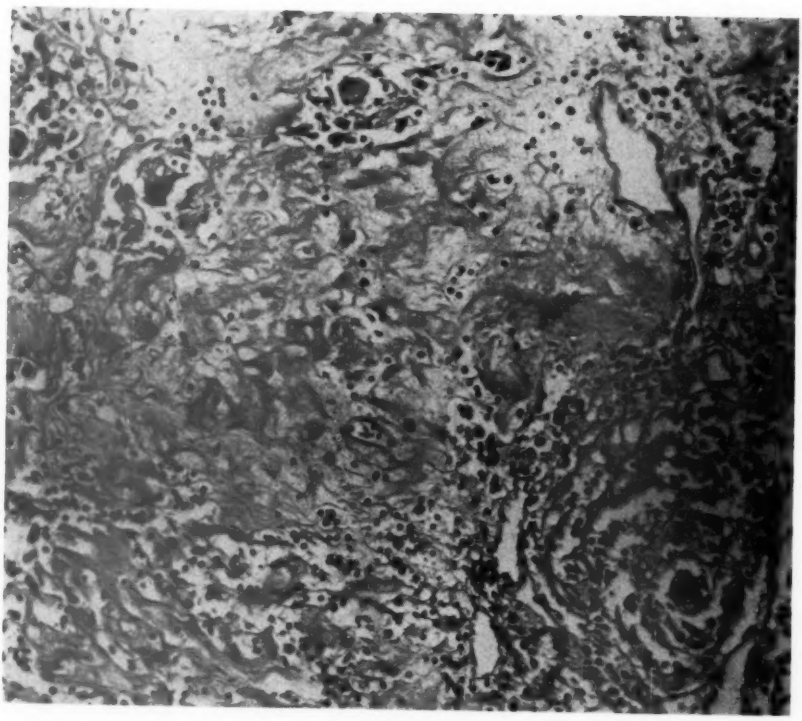
FIG. 12. Case 17. Hyaline fibrosis of lymph nodes with persistence of giant cells. Hematoxylin and eosin stain. × 150.



9 10



11



12

A CLINICOPATHOLOGIC STUDY OF "MIKULICZ'S DISEASE" *

WINFIELD S. MORGAN, M.D., and BENJAMIN CASTLEMAN, M.D.
(*From the Department of Pathology, Massachusetts General Hospital, Boston 14, Mass.*)

In 1888, Johann Mikulicz described at a meeting of the Society for Scientific Medicine in Königsberg the disease, later to bear his name, characterized by bilateral, chronic, painless hypertrophy of the lacrymal and salivary glands. The original case was that of a 42-year-old man in whom, over a period of 7 months, swelling of the lacrymal glands appeared first, followed by swelling in the submaxillary and parotid regions. After removal of two thirds of the lacrymal tumors, swelling reappeared in about 2 months. Further excision of the remaining portions of the lacrymal tumors and the submaxillary glands resulted in improvement, although swelling of the parotid glands persisted. The patient died several months later of probable peritonitis, at which time the swellings were markedly decreased in size.

Mikulicz's paper was published 4 years later.[1] The gross and microscopic features are brought out in the following translated excerpts.[1a]

"The submaxillary glands which had been removed in toto are most deserving of consideration. First of all it must be brought out that each of the glands, swollen to the size of a child's fist, corresponded exactly in relation to form and segmentation into lobes and lobules to the proportions of the normal gland. . . . The tumor shows, in its gross microscopic details, the normal structure of the gland, only it is increased in mass. An essential difference is found, however, by the naked eye in the fresh transverse section in the color and finer structure of the glandular mass forming the individual lobules. In place of the finely granular, gray red structure of the normal gland substance, we see a more homogenous, pale reddish yellow, amyloid mass of lesser transparency. Its consistency is decreased and very fatty. . . .

"The microscopic examination revealed that the main mass of the tumor was a pretty uniformly arranged tissue consisting of small round cells. . . . Here and there the cells lay compactly together; in other places a fine reticulum is to be seen between them. In single, large cells karyokinetic figures can be recognized. Imbedded in these small-celled main masses there appear, partly single and partly in groups, the apparently unchanged acini of the salivary gland; they are separated from one another in varying distances by the round cell tissue.

"Similar relations present themselves in the microscopic examination of the lacrymal gland; only here the acini were found less frequently and, it seemed, were entirely lacking in the outer compressed part of the tumor."

On the basis of the benign course manifested by the patient without evident involvement of the general lymphatic system, subsequent regression of the tumor masses, and on the gross and microscopic findings, Mikulicz concluded that the condition was one of a chronic

* Presented in part before the New England Pathological Society, Boston, February, 1950, and at the Fiftieth Annual Meeting of the American Association of Pathologists and Bacteriologists, St. Louis, April 2, 1953.

Received for publication, October 25, 1952.

low-grade infection. He refused to accept as additional examples of the disease, several cases showing similar bilateral enlargement of the lacrymal and salivary glands, proved to be malignant lymphoma, tuberculosis, and acute inflammation, maintaining that its etiology was of a more obscure nature. Mikulicz did, however, believe that the disease always involved and actually had its origin in the lacrymal glands, involving the salivary glands only at a later stage.

Within a few years, despite Mikulicz's published description and concept of the disease, which in retrospect are clear and definite in many respects, much confusion was precipitated by the appearance in the literature of many cases of bilateral chronic enlargement of the lacrymal and salivary glands, classified as Mikulicz's disease. It soon became apparent that the cause of enlarged lacrymal and salivary glands was not always as obscure as in the case reported by Mikulicz and that the course in some cases was that of a benign, self-limited condition; and in others, a rapidly fatal one. Pathologically, the tissues obtained for biopsy or at necropsy differed widely in various cases.

In 1907, Napp[2] declared the condition to be merely a symptom complex that might be produced by any one of several causes, such as leukemia, malignant lymphoma, atypical lymphomatosis, and tuberculosis. Two years later Howard[3] reviewed 81 cases collected from the literature and grouped them under three headings: (1) Mikulicz's disease proper, (2) malignant lymphoma, and (3) leukemia. This classification was found inadequate by Thursfield[4] who in 1914 made the first attempt to classify the syndrome on an etiologic basis. He divided all cases showing enlargement of the salivary and lacrymal glands into eight groups: a congenital, familial, and hereditary condition; Mikulicz's disease proper; Mikulicz's disease with involvement of the lymphatic apparatus; leukemia; tuberculosis; syphilis; gout; and sialodochitis fibrinosa. In 1927, Schaffer and Jacobsen[5] modified Thursfield's grouping, combining its eight categories into two large groups, Mikulicz's disease proper of unknown etiology, and Mikulicz's syndrome caused by leukemia, lymphosarcoma, tuberculosis, etc.

Although considerable progress was realized when it became evident that bilateral enlargement of the lacrymal and salivary glands was but a syndrome produced by a variety of diseases, an adequate histopathologic study of so-called Mikulicz disease proper is not available.

As will be discussed in other portions of this paper, it is important for purposes of treatment and prognosis that Mikulicz's disease be recognized and separated from other diseases which produce the so-called syndrome and simulate it clinically. Compared to other diagnoses which must be considered, none has been more difficult than the

differential diagnosis of Mikulicz's disease and malignant lymphoma, in both of which the lymphoid tissue is the prominent element. It is not surprising that in the past this has occasioned misdiagnosis or at least some uncertainty as to whether the lesion should be considered benign or malignant.

Because we, too, had experienced this uncertainty in respect to several such cases, we carried out a follow-up study of the patients on whom the diagnosis of malignant lymphoma of the salivary or lacrymal glands had been made, or considered. When this was done, it became apparent that in several of the cases the diagnosis had been correct, the patients having followed the usual course of malignant lymphoma with early death. Six patients, however, were found to be living and free of recurrences 9 to 16 years after surgical removal of the tumor. A study of the histologic sections of these 6 cases disclosed, in addition to diffuse lymphoid infiltration of the gland substance, a characteristic alteration in the duct epithelium, present in all. The thought was entertained that here might be a means of differentiating histologically these cases which in the past had been so confusing.

A further search in our own files and in those of neighboring laboratories provided us with a total of 18 cases which serve as the basis of the present study. In the various laboratories* from which these cases have been collected there had been a wide range of diagnoses. They included: chronic inflammation, Mikulicz's disease, metastatic carcinoma, atypical adenocystoma lymphomatosum, mixed tumor, lymphocytic leukemia, and malignant lymphoma. The last diagnosis was the one most commonly employed. For this reason, a number of these patients were considered to be suffering from malignant disease and treated accordingly.

Clinical Data (Table I)

Most of the patients had a history of a non-tender, progressively enlarging mass in the region of one or more salivary or lacrymal glands. Usually this was associated with no other symptoms, although 4 of the 18 having salivary gland involvement did complain of dryness of the mouth. Three patients who developed secondary infection in the gland experienced a moderate degree of temporary local tenderness. Four patients gave a history of increase and decrease in the size of the mass from time to time and two noticed rather rapid enlargement during the several months just prior to hospitalization.

Fifteen of the 18 patients were female.

* Massachusetts General Hospital, the Massachusetts Eye and Ear Infirmary, Boston City Hospital, New England Deaconess Hospital, Peter Bent Brigham Hospital, and the Harvard Tumor Diagnostic Service.

The ages ranged from 15 to 70 years. Twelve of the 15 women were between 37 and 59 years of age when the disease was first noticed. The ages of the men were 15, 39, and 70 years.

TABLE I

Clinical Data for Eighteen Cases of "Mikulicz's Disease"

Case no.	Age	Sex	Known duration (years)	Gland and location	Number of glands involved	Treatment	Follow up (years)
1	35	F	1	Both parotid glands and both submaxillary glands	4	Surgical excision and x-ray therapy	6
2	61	F	12	Both parotid glands and both submaxillary glands	4	Surgical excision and x-ray therapy	5¾
3	62	F	1/3	Both parotid glands and both submaxillary glands	4	Surgical excision	2
4	39	F	5	Both lacrymal glands	2	Surgical excision	3⅜
5	47	F	1	Both parotid glands	2	None	Necropsy
6	55	F	11	Both parotid glands	2	None	Necropsy
7	70	M	1	Left parotid gland and left submaxillary gland	2	Surgical excision	7
8	59	F	1	Left parotid gland and left submaxillary gland	2	Surgical excision	Necropsy
9	37	F	5/12	Right parotid gland	1	Surgical excision	2/3
10	39	M	1	Right parotid gland	1	Surgical excision	14
11	48	F	1	Right parotid gland	1	Surgical excision, x-ray therapy, and radium seeds	16
12	47	F	1⅜	Left parotid gland	1	Surgical excision	1
13	50	F	10	Left parotid gland	1	Surgical excision	1
14	56	F	2/3	Left parotid gland	1	Surgical excision	13½
15	37	F	5/6	Left parotid gland	1	Surgical excision and x-ray therapy	9½
16	40	F	1/6	Left parotid gland	1	Surgical excision	12
17	69	F	2	Left parotid gland	1	Surgical excision and radium seeds	10
18	15	M	1	Left submaxillary gland	1	Surgical excision	5⅛

The duration of the masses before the patient came to the hospital ranged from 2 months to 12 years. In most cases it was 1 to 2 years. Of 3 long-standing cases, one had enlargement of a single parotid gland for 10 years; another, enlargement of both parotid glands for 11 years; and still another, of both parotid and submaxillary glands for 12 years.

The gland, the number, and the combinations of glands showed remarkable variation in this series. In 9 cases there was involvement of a single parotid gland; in one case, a single submaxillary; in 2 cases, a single parotid and a single submaxillary. There were 3 cases with involvement of both parotid and submaxillary glands, and 2 cases of both parotid glands. In one case the disease was confined to the lacrymal glands. No case was encountered in which all six glands were involved.

A follow-up study ranging from 1 to 16 years was possible on 14 patients who underwent surgery (Table I). In none of these was there clinically any recurrence of the disease locally or spread to other organs. Necropsies of 3 patients, who died of apparently unrelated diseases, failed to show any evidence of malignant lymphoma or of disease in any other organ similar to that present in the salivary glands.

If, for the purpose of follow-up studies, we exclude the 3 patients who died, 15 remain who had surgical excision of one or more of the glandular masses. Of 8 of these patients, on whom specific information was available, one had implantation of radium seeds; another, radium seeds and x-ray therapy; 3 had x-ray therapy; and 3 others were followed in the Out-Patient Clinic with a diagnosis of probable malignancy.

Pathologic Findings

Gross Examination

In most cases, the only abnormal finding at operation was diffuse, symmetric enlargement of the salivary gland (Fig. 1). In others, however, the disease process was focal, giving the involved portions the appearance of nodularity in contrast to adjacent normal gland tissue. When diffusely enlarged, the glands might measure up to 7 by 5 by 4 cm. Except in secondarily infected cases, there was no capsular thickening and the surgical removal was not difficult. The adjacent lymph nodes were not enlarged. The gland retained the normal lobular pattern and general configuration of a salivary gland.

On section, the specimen cut with somewhat rubbery resistance, presenting a surface which was smooth, glistening, and homogeneously pinkish tan. The most striking aspect of the cut surface was the preservation of the lobular architecture and the marked enlargement of the individual lobules, the latter often measuring up to 1 to 2 cm. in greatest diameter. The lobules were separated by thin fibrous septa.

Three specimens in this series contained cystic structures measuring up to 1.5 cm. in diameter, and in one case, thin, tan-brown fluid was described as contents of the cysts.

MICROSCOPIC EXAMINATION

The histologic picture of all 18 cases was so similar as to leave no room for doubt that a single disease process was common to all. This consisted of two co-existing fundamental changes: (1) a gradual lymphoid infiltration and proliferation within the lobule, with subsequent atrophy and loss of acinar tissue until there was complete replacement of the glandular parenchyma by the lymphoid tissue; and (2) an alteration of the ducts, characterized by a typical intraductal cellular proliferation, gradual narrowing of the ductal lumen leading to the formation of a compact cellular island lying in a stroma of lymphoid tissue, and, finally, the deposition of a hyalin-like substance which in time might completely replace the altered duct structures.

Parenchymal Changes

The earliest evidence of involvement of a lobule, in so far as the lymphoid tissue was concerned, was the presence of a scattering of lymphoid cells—usually mature lymphocytes—about one of the intralobular ducts (Figs. 2 and 3). In time, the accumulation of lymphoid tissue became greater, adjacent ducts were surrounded, and groups of immature lymphoid cells might be identified, indicating proliferation. The lymphoid involvement followed a characteristic pattern, first occurring more or less centrally in the lobule and then extending toward the periphery of the lobule until complete replacement of acinar tissue had taken place. When this occurred, the lymphoid tissue, except in rare instances, appeared to remain contained within the lobular septa and did not spread across into adjacent lobules (Fig. 2).

The arrangement of the lymphoid tissue (Figs. 5 and 6) was interesting in that it did not have the appearance of normal lymphatic tissue. Well circumscribed lymph follicles usually were not present. Rather, the numerous aggregations of immature lymphoid cells ordinarily seen in the follicle occurred in nondescript clumps, in streams, or in swirling masses. These were most frequently seen lying adjacent to fibrous trabeculae or about ducts and blood vessels. This lack of discrete follicular organization gave more of an appearance of infiltration, and undoubtedly has been one of the deciding factors responsible for the diagnosis of malignant lymphoma previously made in some of these cases. The picture was further complicated by the presence of moderate to large numbers of similar immature cells scattered throughout the stroma, occurring singly or in clumps of two or three. Many of these cells were in active mitosis. Plasma cells, eosinophils, or polymorphonuclear neutrophils were rare except in cases complicated by secondary infection.

Silver stains (Fig. 16) on sections from cases of about 1 year's duration in which there was complete replacement of the lobule by lymphoid tissue showed a fine background of reticulum similar to that of lymph nodes, but absence of the usual prominent follicular pattern. In cases of longer duration, hyalinization of the stroma similar to that seen in lymph nodes which have been the site of chronic inflammation was very prominent (Fig. 7). The lymphoid cellular infiltration, although less in amount, remained of the same character as that seen in cases of shorter duration.

The changes in the acini were coincident with the advent of the lymphoid infiltration and appeared to be those of simple pressure atrophy (Fig. 8). They first occurred more or less centrally, with gradual and progressive involvement of the lobule as it was replaced by lymphoid tissue. The acini immediately adjacent to the lymphoid tissue became compressed and separated, the nuclei hyperchromatic, and the cytoplasm more darkly acidophilic. As the lymphoid tissue increased in amount the acini became smaller and finally fragmentation and disintegration occurred. There was, in addition, loss of the normally present fat. There were no focal lesions in the acini, and the infiltration by lymphoid cells was wholly periductal. The lymphoid proliferation continued until the entire glandular parenchyma, with the exception of the ducts, was replaced.

Changes in Ducts

Normally, the ducts (Fig. 9) were lined by regular cuboidal or columnar cells with a centrally or basally placed single or double row of rounded nuclei. It has been our experience that when any alteration was noted in a given duct, several of the early changes might be observed. Although the organization of the lining epithelial cells remained intact, the nuclei became hyperchromatic, less regular in outline, and ovoid or spindle-shaped (Fig. 10). The cytoplasm changed from the normally bright acidophilic pink to a more basophilic purple. Later in the early stage, there was an increase and piling up of nuclei with beginning loss of polarity in the position of epithelial cells but also external to these, yet within the basement membrane, in a location which normally would be accorded to myoepithelial cells (Figs. 4 and 11). More advanced stages of alteration were characterized by the presence of a greater number of nuclei of both cell types, apparently indicating that a continued proliferation had occurred. As might be expected, this resulted in a thickening of the layer of cells between the basement membrane and the lumen, and there was subsequent narrowing of the lumen. This cellular proliferation was accompanied by a

prominent disorganization and loss of polarity, so that in a more advanced phase of the early stage the cells appeared to lie in all directions, the "epithelial" layer persisting as a confused mixture of epithelial and myoepithelial cells (Figs. 3 and 4). This disorganization was further complicated and increased by the migration of lymphoid cells into the altered ducts. When this occurred, even greater changes in the cells were observed. There was an obvious loss of cellular cohesion. Many showed hydropic degenerative changes in which pyknotic nuclei of round, ovoid, reniform or spindle shape appeared to lie suspended in vacuolated spaces containing a few scattered acidophilic granules suggestive of remnant cytoplasm. There was considerable variation from one duct to another and although one duct might show the degenerative changes described, another nearby might show none. An additional finding of interest, although not manifest in every duct, was the presence of normal appearing mitotic figures, in some ducts numbering up to twenty.

The proliferation of cells continued, gradually narrowing the lumen until it was completely obliterated. At this time the involved ducts assumed the form of solid, branching, densely cellular cords lying in a stroma or sea of lymphoid tissue (Figs. 12, 13, 14, and 15). Because of this rather striking appearance and for convenience in differentiating these from unaltered ducts, we have elected to call them "epi-myoepithelial islands."

The outline of the epi-myoepithelial islands followed in general that of the ducts from which they obviously arose. In cross section they were roughly circular or ovoid, and on longitudinal section they appeared as straight or branching cords. They were, however, usually one to two times greater in diameter than normal ducts, and occasionally they might reach five to six times the diameter of an intralobular duct and form relatively large, discrete sheets of cells. They were enclosed by an intact basement membrane which was usually visible with the ordinary stains, but particularly well shown with silver stains (Fig. 16).

If one excluded the migrating lymphoid cells which usually were present in varying numbers, the islands were composed of two types of cells, epithelial and myoepithelial. Their separation into two distinct cell types, however, was not easy. Because of the disorganization and indefinite cell borders the distinction was made almost wholly on the character of the nuclei. We recognized as epithelial those cells in which the nuclei were fairly large and ovoid, circular, or irregularly reniform. These nuclei were generally vesicular with a fine scattering of clumped or granular chromatin. In contrast, the myoepithelial cells

were thin and elongated with prominent, usually hyperchromatic, spindle-shaped nuclei, on cross section triangular, with rather poorly defined, fibrillar, acidophilic cytoplasm (Fig. 12). Usually it was only when they were present at the periphery of the island that the myoepithelial cells could be distinguished from the lymphoid cells and identified with any degree of certainty. Attempts to distinguish these cells by means of special stains, including the Masson erythrosin-saffron stain employed and recommended by Kuzma[6] in his study of breast tumors, were unsuccessful.

Following the intraductal cellular proliferation, the obliteration of the duct lumen, and the formation of an epi-myoepithelial island, a second characteristic change, the deposition of hyalin (Figs. 13 and 14) occurred.

Here, the hyalin, as seen elsewhere in the body, was homogeneous and brightly acidophilic. It was of interest that in the early stages of development, it appeared to occur in two rather distinct positions in regard to its relationship with the cells composing the island. When in the form of droplets or globules, one or more of the hyperchromatic nuclei lay against the edge of the material (Figs. 13 and 14) and a thin membrane, which appeared also to include the nucleus, extended around the droplet's edge. This was especially well demonstrated by use of silver stains and strongly suggested that the material was intracellular, the nucleus being pushed to one side. Elsewhere, however, the hyaline material seemed to thread its way between the cells and appeared to be extracellular. Thus, in a given island, the exact positional relationship of the cells with the hyaline material was just as indefinite as that existing between one cell and another.

A very definite correlation existed between the degree of duct alteration and the duration of the disease clinically. This was of such degree as to permit one to estimate with considerable accuracy from a given slide what the approximate duration of the disease might be. Those cases in which the disease had been clinically active for about 1 year showed minimal deposition of hyaline material, those active up to 5 years, moderate deposition; while cases with active symptoms for 10 years or more, exhibited severe hyaline changes in which there was often essentially complete replacement of the islands so that they persisted throughout the stroma as scattered, densely acidophilic, almost acellular bodies (Fig. 7).

Examination of many of the epi-myoepithelial islands disclosed that there was no uniformity from one island to the next in the proportion of myoepithelial to epithelial cells, one showing epithelial cells in predominance, another myoepithelial, and still another, a more even mix-

ture. There was evidence to suggest that those islands which contained a greater number of myoepithelial cells were associated with a greater deposition of hyalin, while those in which there was a predominance of epithelial cells showed attempts at formation of duct-like structures which contained acidophilic granular material which differed from hyalin and had the staining reactions of duct secretions.

Some intralobular and interlobular ducts showed a change in the epithelium which was similar to that previously described for the smaller ducts, except that the process did not go on to obliteration of the lumina. In contrast, these were frequently dilated (Fig. 2) and it seemed most probable that it was by cystic dilatation of larger ducts that cysts of macroscopic size were produced in several cases. The epithelium showed proliferative activity, varying from three to ten cells in height. It might in areas be thrown into small projections, although these in no way resembled the true papillary formations such as are seen in papillary adenocystoma lymphomatosum. In the larger cysts, the lining cells were supported by an irregular layer of dense, homogeneous, usually acellular, hyaline connective tissue. Occasionally a cystic structure was seen to be connected with an epi-myoepithelial island. The dilated ducts or cysts might contain masses of lymphocytes, acidophilic amorphous matter or needle-like crystals, or small, rounded, bluish, calcified bodies. In no instance were cholesterol crystals identified.

The Lacrymal Gland

Although little has been said specifically about the lacrymal glands, the gross and microscopic findings in the one case in this series were very similar to those just described for the salivary glands.

Selective Review of the Literature

An attempt to find in the literature other cases with similar microscopic findings met with only slight success. Scattered reports during the past 30 years have called attention to diffuse lymphoid infiltration of the salivary glands associated with duct epithelial changes. In most of these articles, however, the latter aspect of the lesion was considered of minor importance, emphasis being placed on the lymphoid hyperplasia. Follow-up studies in most cases suggested disease of chronic type, but opinions differed as to whether or not the lesion was benign or malignant. No large series of cases in which definite conclusions were reached was found.

Smith and Bump,[7] in 1928, reported a case of a 62-year-old woman who was admitted because of bilateral parotid swellings of approxi-

mately 1 year's duration. At operation, a reddish encapsulated mass was removed from the region of the left parotid gland. The initial histologic report was "lymph node containing metastatic carcinoma which in places had a duct-like appearance." A subsequent diagnostic consideration by this same observer was "embryonal carcinoma, possibly from the remains of a branchial cleft." The final diagnosis rendered after consultation with another pathologist was Mikulicz's disease. Although these authors drew attention to the ductal epithelial changes and stated that by serial sections they had shown these changes to extend along the duct system, they believed the duct alteration to be squamous metaplasia and destruction due to infiltration by lymphocytes.

Sidahara,[8] in 1937, reported a case in a middle-aged man of lymphosarcoma of the parotid gland cured by surgical removal. His photomicrographs show very clearly the duct changes and epi-myoepithelial islands so characteristic of the lesion being described.

In 1938, Swinton and Warren,[9] reviewing their series of parotid gland tumors, found 7 cases which they considered of particular interest from the standpoint of differential diagnosis. All of these cases occurred in women, with an average age of 55.9 years. The histologic picture described is consistent with that presented in this paper; however, these authors also considered the duct change to be squamous metaplasia. Follow-ups of 1 to 6 years on these cases showed no evidence of recurrence in any case and the authors believed the lesion to be a benign, chronic inflammatory process.

Lehman and Leaman,[10] in 1940, reported a case of a 55-year-old woman who complained of sore eyes for 3 years and dryness of the mouth for 2 years. Physical examination disclosed swellings over the regions of both lacrymal glands and one salivary gland. These authors considered the lesion to be Mikulicz's disease proper. Although they did not emphasize it, the typical alteration in the duct epithelium was evident in the photomicrographs.

In 1942, Skorpil[11] presented in a paper on benign lymphoma of the salivary glands an account of 2 cases of unilateral parotid swelling occurring in a 15-year-old boy and a 34-year-old woman which had been followed for 3 and 16 months, respectively. He described the duct changes in detail and, although he found no definite cause, he believed the pathologic changes to be most probably of infectious origin.

Of these 11 cases, all but 2 were of females, and all but the 15-year-old boy reported by Skorpil[11] fell into the age group of the fourth to seventh decade.

Differential Diagnosis

1. Chronic Inflammation (Figs. 17 and 18). The illustrations show the usual form of chronic inflammation seen in the salivary glands, frequently associated with calculi. Grossly there is fibrosis, atrophy, and distortion of lobular architecture. Microscopically, the fibrosis, loss of acinar tissue, and infiltration by chronic inflammatory cells composed of plasma cells, lymphocytes, and eosinophils serve to differentiate the two diseases. In addition, the ducts which persist usually show changes quite different from those seen in Mikulicz's disease (Fig. 18). They may occasionally show squamous metaplasia which, again, is readily distinguished from the characteristic ductal epithelial change previously described. Sixty cases of chronic inflammation of the salivary glands were studied as control material; in none were epi-myoepithelial islands found.

2. Adenocystoma Lymphomatosum (Fig. 19). Adenocystoma lymphomatosum should not offer much difficulty in differential diagnosis. Grossly, it has a characteristic appearance, and, in contrast to the features previously ascribed to Mikulicz's disease, occurs as a discrete, encapsulated, roughly rounded, soft or cystic mass lying within or protruding from the substance of the gland. Microscopically, the typical tall, eosinophilic, columnar epithelium with a double row of nuclei, the usual papillary character, and the lymphoid stroma with well formed follicles, readily serve to differentiate the two. Fifteen cases of adenolymphoma from our own files were studied as control material.

3. Metastatic Carcinoma. Metastatic carcinoma is included as a possibility only for emphasis because this diagnosis was rendered on one case in this series and in one found in the literature. Although it should be possible to differentiate the two in permanent sections, one could readily imagine that a pathologist unfamiliar with the lesion, when presented with a few fragments for frozen section in which the epi-myoepithelial islands were prominent might easily give this diagnosis serious consideration (Fig. 15).

4. Malignant Lymphoma. Of the several diseases from which Mikulicz's disease must be differentiated microscopically, the most important one is malignant lymphoma (Figs. 20 and 21), for it is to this condition that it bears the greatest resemblance. Ten cases of malignant lymphoma of the salivary gland proved by necropsy or subsequent lymph node biopsy were studied.

In contrast to Mikulicz's disease in which preservation of lobular architecture is a characteristic feature grossly, in the cases of malig-

nant lymphoma the prominent finding is that of a fairly discrete, nodular tumor in a portion of the gland in which the lobular architecture is absent. Three differences found histologically were as follows: (1) Whereas in the benign disease, the lymphoid tissue is with rare exceptions contained within the lobule and the interlobular septa are preserved, in malignant lymphoma the cells soon pass in all directions from one lobule to the next and septa are obliterated. (2) The cell type, as in the stem cell and Hodgkin's lymphomas,[12] will undoubtedly provide a measure of security in the diagnosis, and it is probable that only in the lymphocytic type of malignant lymphoma will there be a real danger of misdiagnosis. Although 17 of the 18 cases in this series showed a predominantly mature lymphocyte type composing the stroma, there was one case in which immature cells of the lymphoid series occurred in considerable numbers. In addition, many cells with double nuclei were present which could be justifiably considered atypical. Sections from this case were shown to several competent pathologists and each made a diagnosis of malignant lymphoma. However, typical epi-myoepithelial islands were present, and in view of what would otherwise represent a clinical cure of malignant lymphoma, we have little hesitation in including the case in the benign group. In several other cases the number of lymphoid cells in active mitosis was great. Although this point must await confirmation by the study of additional cases, it seems worth while at this time to suggest that in some cases the degree of lymphoid hyperplasia may be great and the character of the cells disturbing. (3) The typical epithelial changes of the ducts with the formation of the epi-myoepithelial islands, in our experience, has been the most dependable way of distinguishing the two diseases. In none of the cases of malignant lymphoma were these changes found. In lobules involved by malignant lymphoma the acinar tissue is often replaced, but aside from perhaps slight atrophy the ducts usually remain, with essentially unaltered epithelium.

Although it would appear, from this study, that the presence of the characteristic ductal epithelial change with the formation of the epi-myoepithelial islands is unique and probably pathognomonic of Mikulicz's disease, it seems prudent to suggest that until additional cases of this disease are studied, a conservative approach to the diagnosis, utilizing all three of the differential points, be employed.

Discussion

In considering the possibilities of pathogenesis in this disease it was necessary to determine, if possible, which of the two prominent findings histologically—the ductal epithelial change or the lymphoid tis-

sue—was the fundamental defect. This was difficult for several reasons. First, changes in the ducts are not observed in the absence of at least minimal lymphoid infiltration, and second, the lymphoid element is by far the more impressive.

Although we have only morphologic evidence, it is our opinion that the primary lesion in this disease is an involvement of the duct system, manifested by the duct changes previously described, and that the lymphoid tissue is simply a secondary response evoked by that basic defect. The following observations support this contention: (1) In no other disease of the salivary or lacrymal glands has the typical ductal epithelial change been found; whereas, the lymphocyte is commonly seen in chronic inflammation. Well developed follicles in close relation to ducts and heavy infiltration of the duct epithelium by inflammatory cells are frequent in the usual case of chronic inflammation. It seems reasonable to expect that this must produce considerable trauma to the duct epithelium and is possibly an agent in the development of squamous metaplasia. The fact that this occurs in chronic inflammation and does not result in a change in the duct epithelium similar to that found in Mikulicz's disease is strongly suggestive that it is not the migration of lymphoid cells into the epithelium over a relatively long period of time which effects the characteristic changes noted. (2) The character of the lymphoid tissue, which appears to respond to certain growth controls to the extent that lobular architecture is preserved, is more in keeping with a response of a secondary nature in contrast to an uncontrolled invasive one, such as that seen in malignant lymphoma. (3) The spotty, but frequently observed involvement, in which a normal lobule may be immediately adjacent to a lobule which is partially or completely replaced by lymphoid tissue (Fig. 2), and the varying amounts of lymphoid tissue, usually in proportion to the ductal epithelial change and confined to the periductal area, are, again, more than suggestive that the disease occurs primarily in the ductal system, involving some radicals and sparing others. Relative to this point, it is of particular significance that Johnson and Goodpasture,[13] in their cases of mumps parotitis produced experimentally by injection of infectious material into Stensen's duct in monkeys, were similarly impressed by the irregular, focal involvement of lobules.

Arguments in favor of the lymphoid tissue being the primary defect are (1) the observation that the infiltrate is composed almost solely of cells of the lymphocytic series, a finding with which we cannot make an analogy in any other benign disease process except Hashimoto's struma of the thyroid gland, (2) the character of the lymphoid tissue with its

lack of organization into a discrete follicular pattern as is usually the case in other conditions in which lymphoid tissue represents an inflammatory reaction, and (3) the observation that in many of the ducts in the early stages, before the epi-myoepithelial island is formed, the epithelium shows marked distortion and disorganization, and the lumen is filled with masses of lymphocytes, indicating the migration of the lymphocytes through the duct wall into the lumen.

An attempt to determine the etiology of this disease was unsuccessful. Most patients were clinically well aside from the asymptomatic enlargement of their salivary or lacrymal glands. There was no evidence of a familial tendency nor for explanation on the basis of exposure to toxic materials. Syphilis, which had been considered in some of the earlier case reports, seemed justifiably ruled out on the basis of negative serologic tests on 12 of the patients in this series. Avitaminosis, which again was considered by some authors, could not be confirmed by a study of the social histories of our own cases. These cases differed clinically from the usual ones in which chronic inflammation and calculus are found. In no case did a severe episode of pain on swallowing bring the patient to the hospital, and in none were calculi of more than microscopic size found. In none of the patients were the initiating symptoms those of acute inflammation, although as stated earlier, several cases did become secondarily infected and were then characterized by episodes of redness, pain, and swelling. Histologically, there was no evidence of granuloma formation. The lack of a strong allergic history clinically and the absence of eosinophils histologically tended to rule against this lesion being of the more usual type of allergic origin. In neither histologic sections nor in smears of fluid expressed from ducts in several cases was there evidence of a parasitic etiology. A careful search of numerous sections from all cases for an alteration suggestive of viral etiology, such as the giant cells in the duct epithelium described by Farber and Wolbach,[14] was unsuccessful, and no relationship to mumps parotitis could be detected.

Since the etiologic mechanism in this disease was not established during the course of study, we are left to consider the clinical and morphologic findings which did result with the hope that these may provide information and some better understanding of the nature of the disease.

That the disease is benign and chronic in character appears clearly evident from the long clinical observation of most of the cases in this series and the detailed examination of the 2 cases which were necropsied. That it differs, however, from the usual forms of chronic inflammation is made apparent by the distinctive clinical history and the

gross and microscopic appearances. These observations, plus the rarity of the disease and the frequent bilateral glandular involvement, would tend to lead one away from the possibility of duct obstruction, the most common offending mechanism in chronic inflammation, as being the etiologic agent also in Mikulicz's disease.

The fact that 15 of the 18 cases (83.3 per cent) and 9 of the 11 (81.8 per cent) found in the literature occurred in women is of more than passing interest. This sex incidence is indeed at variance with that of the usual form of chronic inflammation, which is more frequent in males. It is difficult to know what significance, if any, should be attached to the fact that a majority of the female patients developed the disease at about middle age. An analysis of the time relationship of the menopause and the onset of the disease in the women of this series demonstrated no significant correlation, since the onset occurred both before and after the menopause.

This tendency for the disease to occur in middle-aged females would provide an interesting problem for the endocrinologist were it not for the presence in the current series of 3 cases, about which there could be no doubt, occurring in males aged 15, 39, and 70 years. The physical examinations of these individuals, 2 of whom were seen in the hospital at relatively frequent intervals, failed to reveal evidence of any endocrine abnormality.

Taken separately, the age and sex incidence shown by this disease appears to offer little in way of explanation. When considered together, however, and in combination with the microscopic finding of diffuse lymphoid infiltration, the intriguing possibility of some relationship to Hashimoto's struma of the thyroid is presented. However, examination of the clinical histories of the cases in our series as well as those found in the literature failed to support this possibility, since in no case was there a history of thyroid disease either before or at the time of admission for salivary glandular swelling. Further, Statland, Wasserman, and Vickery,[15] examining 51 patients with Hashimoto's struma from this hospital, found only one who had swelling of the salivary glands.

In considering other diseases which manifest enlargement of the salivary glands, a series of papers by Sjögren[16-25] describing a condition consisting of keratoconjunctivitis sicca, bucco-pharyngo-laryngitis sicca, swelling of the salivary glands, and polyarthritis, usually of the rheumatoid type, were encountered. The possibility of a relationship between Mikulicz's disease and this syndrome appeared more likely

when it was learned that Sjögren's syndrome also occurred almost exclusively in women past the age of 40 years. It was strengthened still further by Sjögren's description of the histopathology of the parotid gland in which lymphoid infiltration and duct changes are the prominent findings. One of Sjögren's photomicrographs[22] (p. 76) depicts an epi-myoepithelial island lying in a stroma of lymphoid tissue. In view of the similarity of some of the clinical and pathologic aspects of these two diseases, our material is being re-investigated. The results of that study will be reported separately.

Summary and Conclusions

The clinical and pathologic aspects of Mikulicz's disease have been studied in a group of 18 cases.

The disease is benign and chronic and may involve one or more salivary or lacrymal glands. At variance with Mikulicz's original belief that the disease is always bilateral and always involves the lacrymal glands, it has been found that the disease is frequently confined to only one salivary gland and affects the lacrymal glands less often than it does the salivary glands.

It occurs predominantly in women, in the fifth and sixth decades.

Grossly, the disease is characterized by preservation of the normal lobular architecture and diffuse enlargement, features which tend to distinguish it from other diseases of the salivary or lacrymal glands.

Histologically, the disease is characterized by replacement of the acinar parenchyma by lymphoid tissue and an intraductal proliferation of two cell elements, epithelial and myoepithelial, with the formation of epi-myoepithelial islands. The presence of the latter offers the most dependable means by which Mikulicz's disease may be distinguished from malignant lymphoma with which it has most often been confused.

On the basis of certain clinical and pathologic similarities to Sjögren's syndrome, it seems likely that Mikulicz's disease is not a distinct clinical and pathologic disease entity as previously believed, but merely one manifestation of a more generalized symptom complex known as Sjögren's syndrome. This possibility is being studied and if confirmed, a further relationship to rheumatoid arthritis and associated diseases is strongly suggested.

We wish to express our appreciation to Drs. Frederic Parker, Jr., Shields Warren, William Meissner, Olive Gates, and Parker Heath for granting permission to use their cases in this study. Most of these individuals also reviewed the slides on these 18 cases and offered valuable suggestions. We wish also to acknowledge our debt to the late Dr. Tracy B. Mallory for his opinions, interpretations, and kindly advice in the early stages of this work.

REFERENCES

1. Mikulicz, J. Über eine eigenartige symmetrische Erkrankung der Thränen- und Mundspeicheldrüsen. Beitr. z. Chir. Festschr. f. Theodor Billroth, Stuttgart, 1892, pp. 610–630.
1a. Mikulicz, J. Concerning a peculiar symmetrical disease of the lacrymal and salivary glands. *M. Classics*, 1937–38, **2**, 165–186.
2. Napp, O. Ueber die Beziehungen der Mikuliczschen Erkrankung zur Tuberculose. *Ztschr. f. Augenh.*, 1907, **17**, 513–517.
3. Howard, C. P. Mikulicz's disease and allied conditions. *Internat. Clin.*, 1909, **1**, 30–63.
4. Thursfield, H. Bilateral salivary swellings (Mikulicz's disease): a clinical review. *Quart. J. Med.*, 1913–14, **7**, 237–249.
5. Schaffer, A. J., and Jacobsen, A. W. Mikulicz's syndrome. A report of ten cases. *Am. J. Dis. Child.*, 1927, **34**, 327–346.
6. Kuzma, J. F. Myoepithelial proliferations in the human breast. *Am. J. Path.*, 1943, **19**, 473–489.
7. Smith, J. F., and Bump, W. S. Lymphoid hyperplasia of lacrymal and salivary glands; Mikulicz's disease. *Ann. Surg.*, 1928, **88**, 91–97.
8. Sidahara, G. Ein operativ geheilter Fall von Lymphosarkom der Parotis. *Gann*, 1937, **31**, 41–49.
9. Swinton, N. W., and Warren, S. Salivary gland tumors. *Surg., Gynec. & Obst.*, 1938, **67**, 424–435.
10. Lehman, J. A., and Leaman, W. G., Jr. Mikulicz' disease. *Internat. Clin.*, 1940, **3**, 105–111.
11. Skorpil, F. Über das benigne Lymphom der Ohrspeicheldrüse. *Frankfurt. Ztschr. f. Path.*, 1941–42, **56**, 514–533.
12. Gall, E. A., and Mallory, T. B. Malignant lymphoma. A clinico-pathologic survey of 618 cases. *Am. J. Path.*, 1942, **18**, 381–429.
13. Johnson, C. D., and Goodpasture, E. W. The histopathology of experimental mumps in the monkey, *Macacus rhesus*. *Am. J. Path.*, 1936, **12**, 495–510.
14. Farber, S., and Wolbach, S. B. Intranuclear and cytoplasmic inclusions ("protozoan-like bodies") in the salivary glands and other organs of infants. *Am. J. Path.*, 1932, **8**, 123–135.
15. Statland, H., Wasserman, M. M., and Vickery, A. L. Struma lymphomatosa (Hashimoto's struma). Review of fifty-one cases with a discussion of the endocrinologic aspects. *A. M. A. Arch. Int. Med.*, 1951, **88**, 659–678.
16. Sjögren, H. Zur Kenntnis der Keratoconjunctivitis sicca (Keratitis filiformis bei Hypofunktion der Tränendrüsen). *Acta ophth.*, 1933, suppl. 2, 151 pp.
17. Sjögren, H. A New Conception of Keratoconjunctivitis Sicca (Keratitis Filiformis in Hypofunction of the Lacrymal Glands). (Translated by J. Bruce-Hamilton.) Australasian Medical Publishing Company, Ltd., Glebe, N.S.W., Australia, 1943, 152 pp.
18. Sjögren, H. Zur Kenntnis der Keratoconjunctivitis sicca. II. Allgemeine Symptomatologie und Ätiologie. *Acta. ophth.*, 1935, **13**, 1–39.
19. Sjögren, H. Zur Kenntnis der Keratoconjunctivitis sicca. III. Mikroskopische Veränderungen der Nasendrüsen. *Acta ophth.*, 1935, **13**, 40–45.
20. Sjögren, H. Dry keratoconjunctivitis as partial symptom of larger symptom complex. *Nord. med. tidskr.*, 1935, **9**, 953–960.
21. Sjögren, H. Further studies of keratoconjunctivitis sicca. *Acta ophth.*, 1937, **15**, 519–520.
22. Sjögren, H. Zur Kenntnis der Keratoconjunctivitis sicca. IV. Mikroskopische Untersuchungen über das Initialstadium der Drüsenveränderungen. *Acta ophth.*, 1938, **16**, 70–79.

23. Sjögren, H. Zur Kenntnis der Keratoconjunctivitis sicca. V. Augenveränderungen nach Exstirpation der Tränendrüse Behandlung. *Acta ophth.*, 1938, **16**, 80–88.
24. Sjögren, H. Zur Kenntnis der Keratoconjunctivitis sicca. VI. Das Siccasyndrom und ähnliche Zustände. Dacryo-Sialo-Adenopathia atrophicans. *Acta ophth.*, 1940, **18**, 369–382.
25. Sjögren, H. Keratoconjunctivitis Sicca. In: Ridley, F., and Sorsby, A. (eds.) Modern Trends in Ophthalmology. Butterworth & Co., Ltd., London, 1940, 403–413.

[*Illustrations follow*]

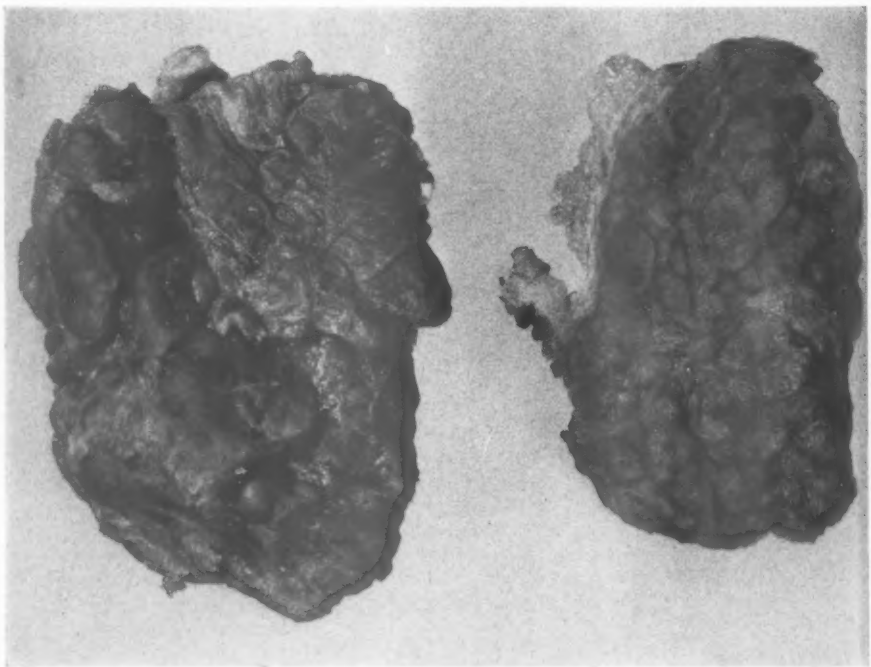
LEGENDS FOR FIGURES

Figures 1 to 4 and 6 to 16 inclusive are of "Mikulicz's" disease.

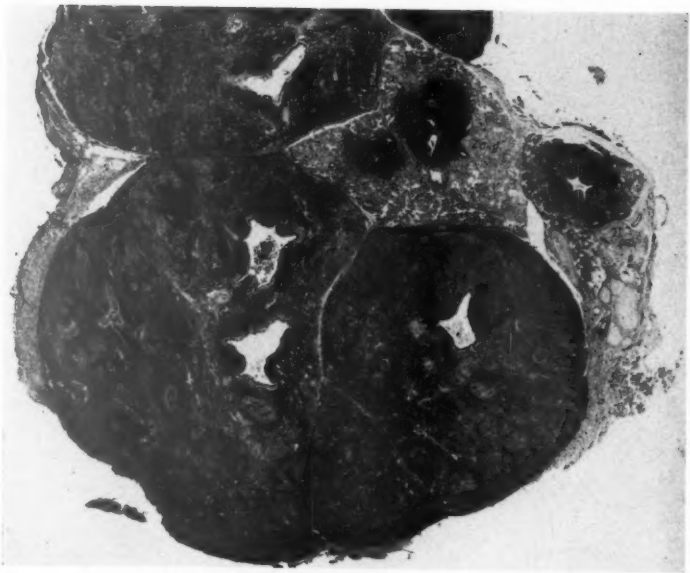
FIG. 1. Parotid gland (actual size) showing the typical gross appearance. The preservation of lobular architecture and enlargement of individual lobules may be noted.

FIG. 2. Section showing lobular preservation and the sharp confinement of lymphoid tissue to certain lobules. The darker appearing lobules or portions of lobules are those in which the acinar parenchyma has been replaced by lymphoid tissue. Dilated ducts and scattered epi-myoepithelial islands are present. × 9.

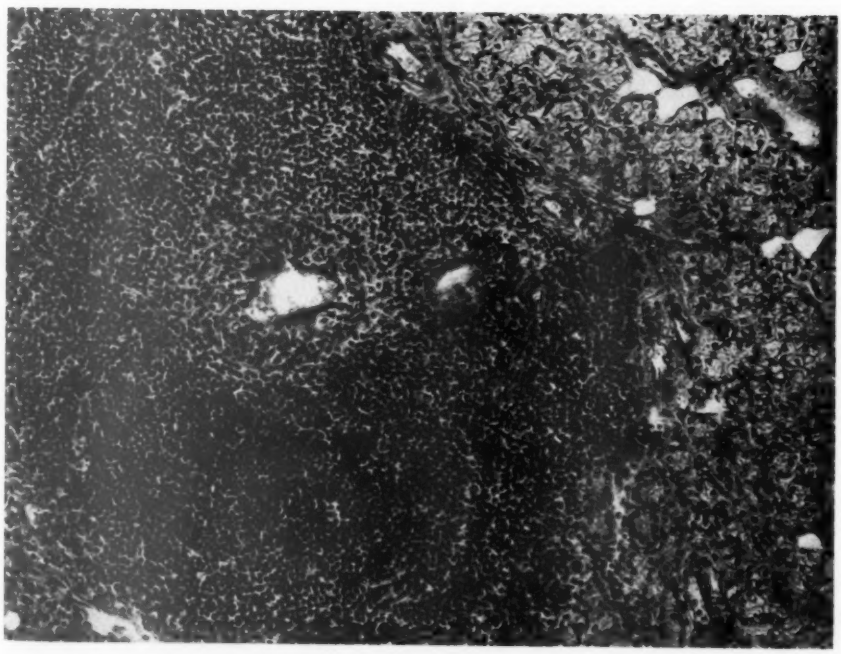
FIG. 3. Area showing early periductal lymphoid infiltration and adjacent normal glandular parenchyma. There is disorganization of the epithelium in the centrally placed ducts. For comparison with normal duct in upper right corner. × 100.



1



2

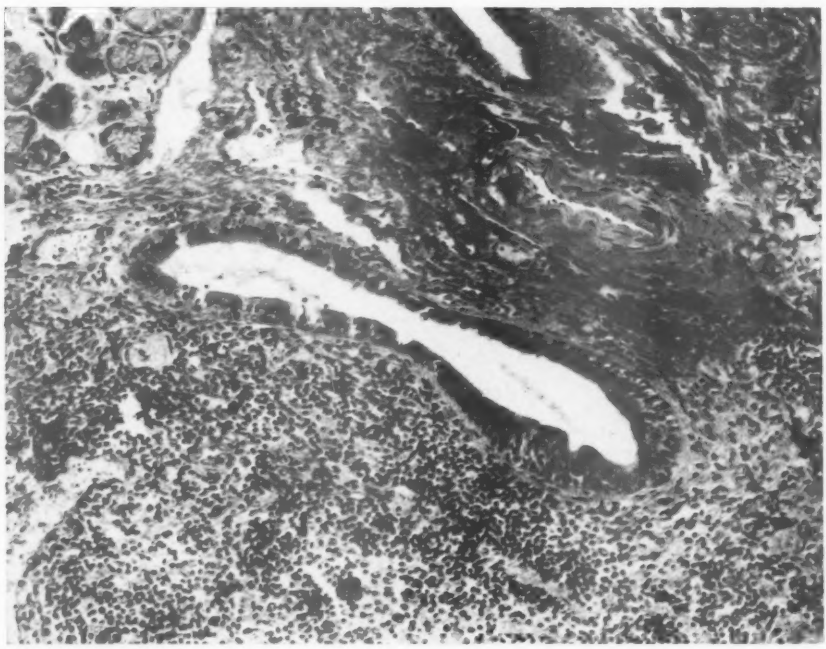


3

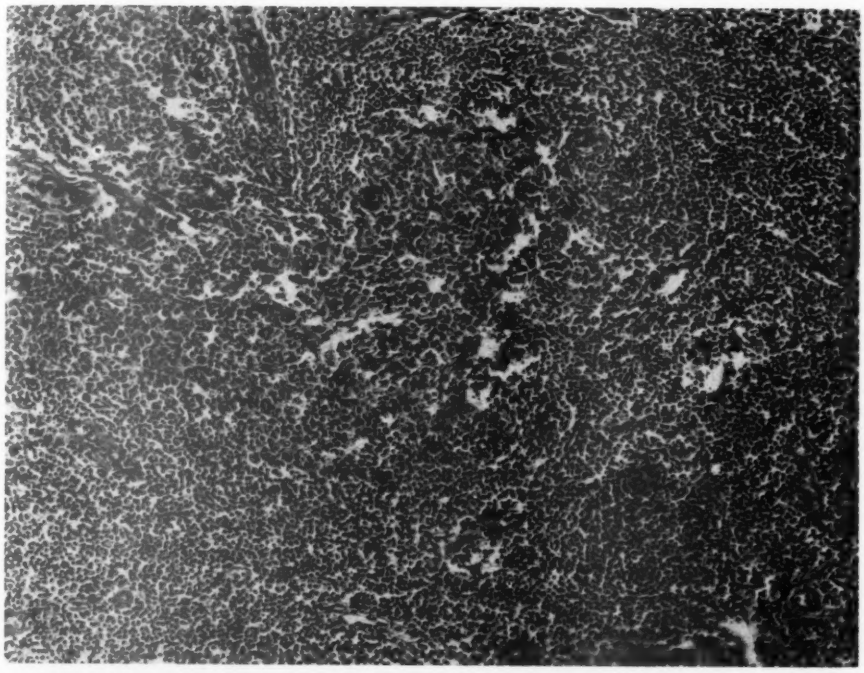
FIG. 4. A duct showing partial change. The left half has normal epithelium composed of cells of uniform size, shape, and polarity with relatively pale-staining cytoplasm. In the right half, the epithelium shows piling-up of cells, poor definition of cell membranes, variation in size and shape, distinct disproportion of nuclear size to the amount of cytoplasm, and loss of polarity. × 100.

FIG. 5. Section showing the character of the lymphoid stroma. × 180.

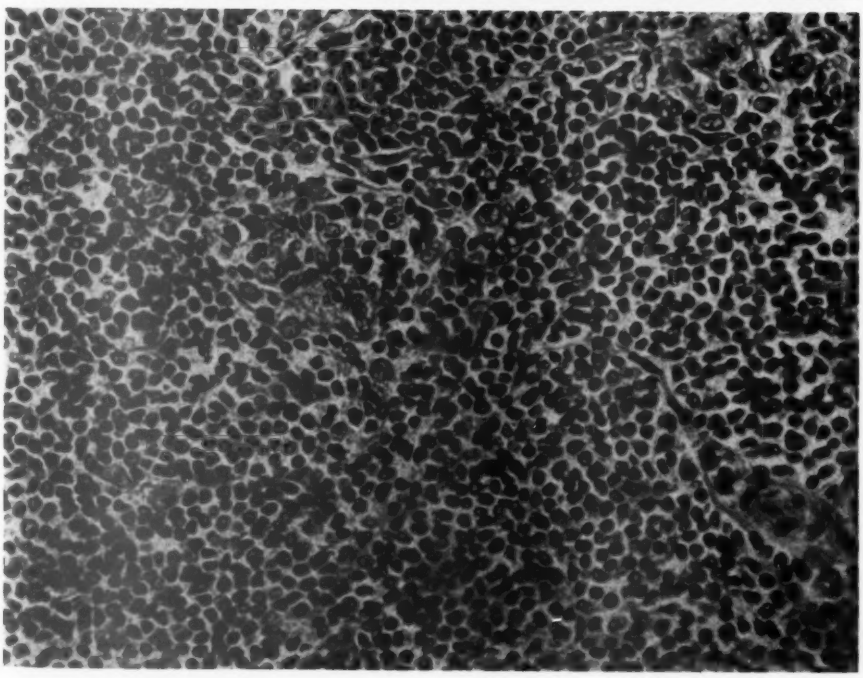
FIG. 6. Higher magnification of another area of the lymphoid stroma. × 400.



4



5



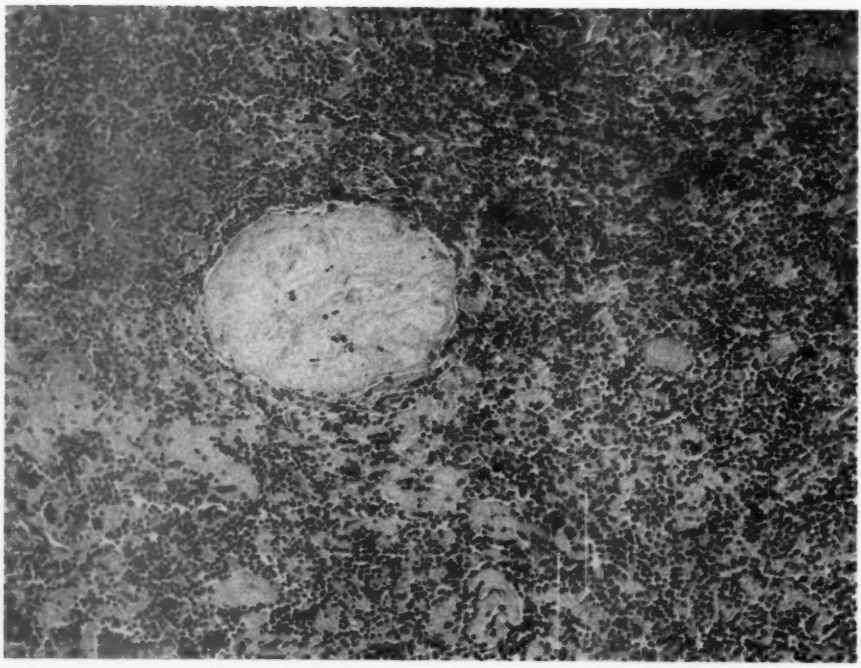
6

FIG. 7. From a case in which the disease had been active for 10 years. Of note are the hyalinization of the stroma and an epi-myoepithelial island. × 180.

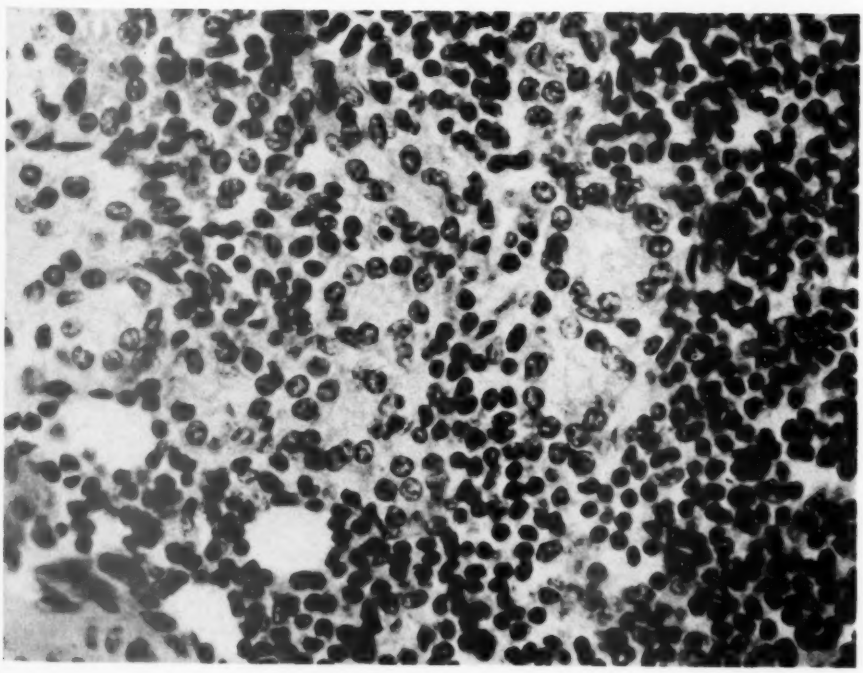
FIG. 8. Area showing separation and atrophy of acini due to infiltration by lymphoid tissue. × 400.

7

8



7



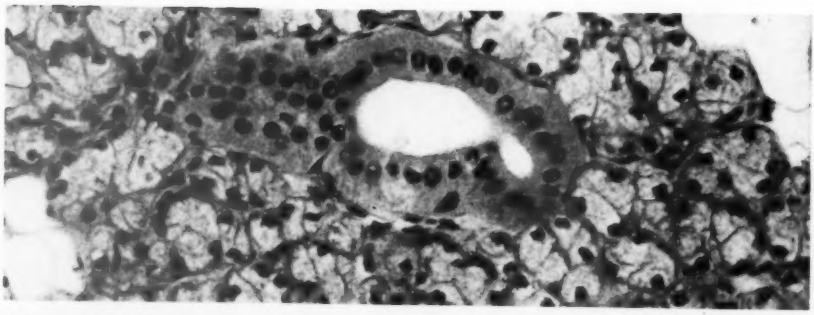
8

FIG. 9. Section of normal salivary gland showing a normal duct, acinar tissue, and fat. × 400.

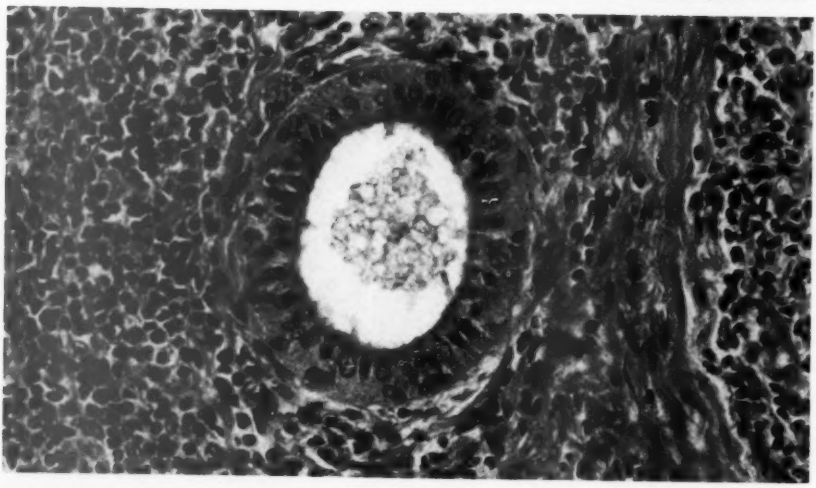
FIG. 10. Duct showing very early change. There is a slight increase in the number of cells of the epithelial layer, especially in the basal region. × 400.

FIG. 11. A duct showing advanced alteration characterized by an increase in the number of cells, loss of polarity, disproportion of nuclear size to cytoplasm, and narrowing of the lumen. × 400.

9



10



11

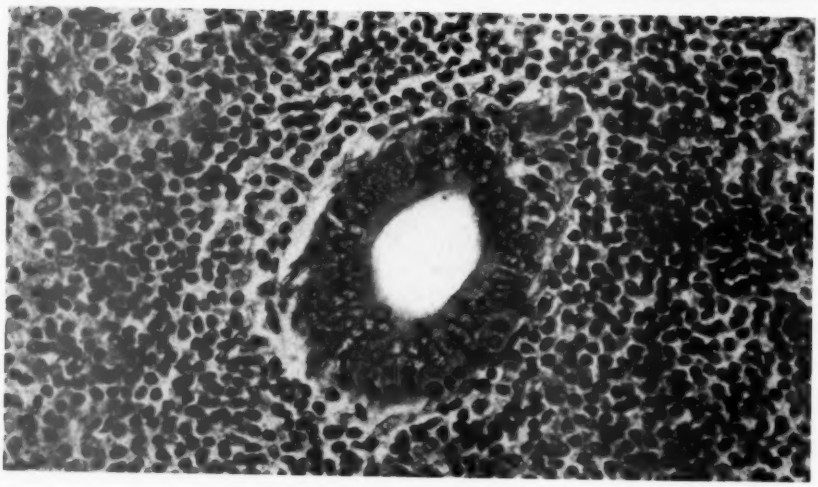
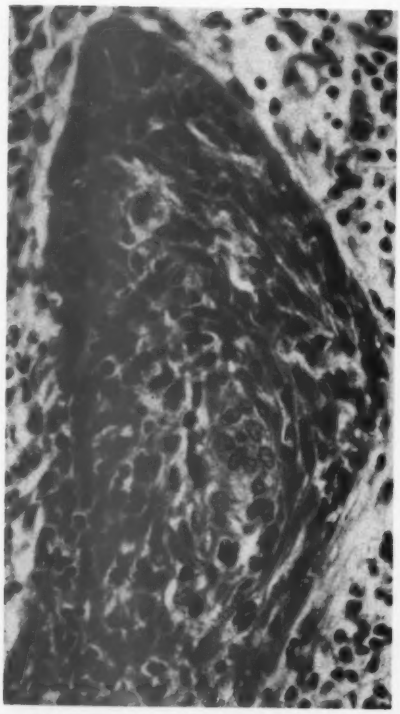


FIG. 12. Section showing an epi-myoepithelial island. Of note are the poor definition of cell membranes, the loss of purposeful arrangement, and the two types of cells (see text). × 400.

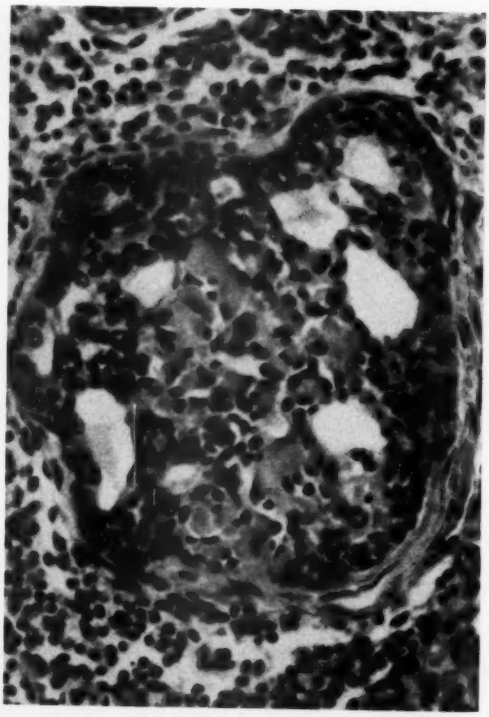
FIG. 13. Another epi-myoepithelial island showing early hyalinization centrally. × 400.

FIG. 14. Epi-myoepithelial island showing duct-like structures and hyalin. × 400.

12



14



13

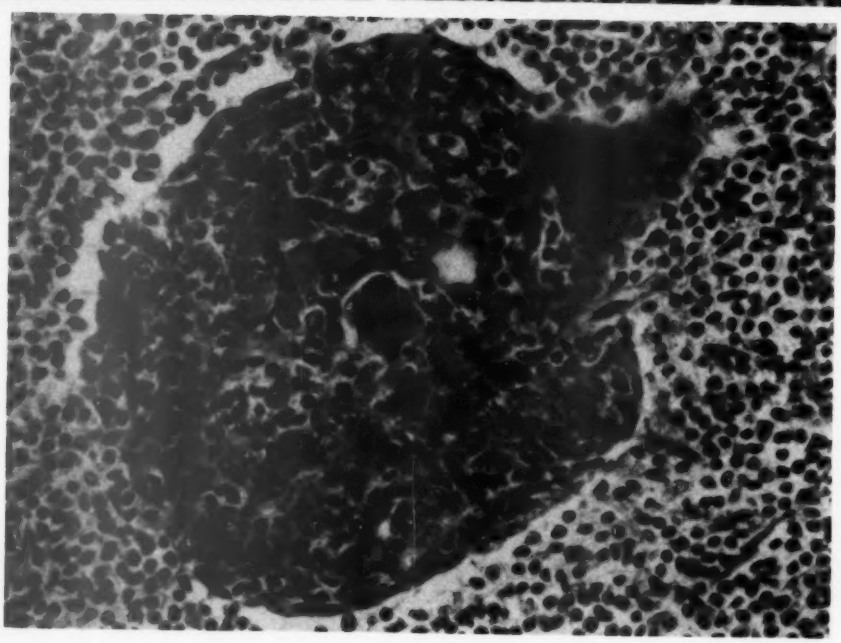
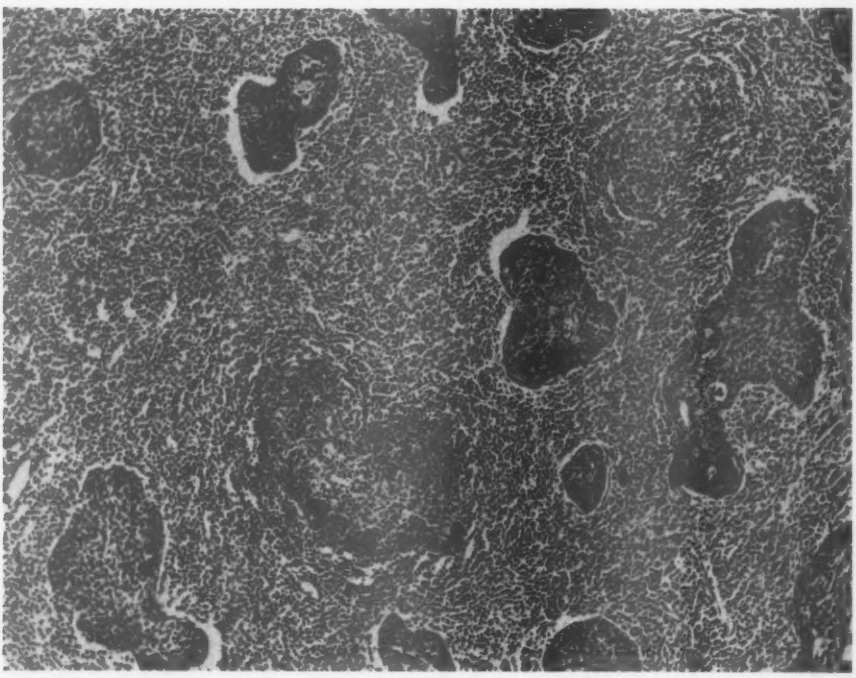


FIG. 15. Area showing scattered epi-myoepithelial islands in a lymphoid stroma. × 180.

FIG. 16. A Foot stain showing the fine background of reticulum in the stroma and the preservation of basement membrane in an epi-myoepithelial island. × 180.

FIG. 17. Chronic sialadenitis. An example of the type of lesion ordinarily seen in duct obstruction. × 180.

FIG. 18. Chronic sialadenitis showing the typical appearance of the ducts in this condition. Dilatation of the lumen and flattening of the ductal epithelium are in contrast to the lesion described in Mikulicz's disease. × 400.

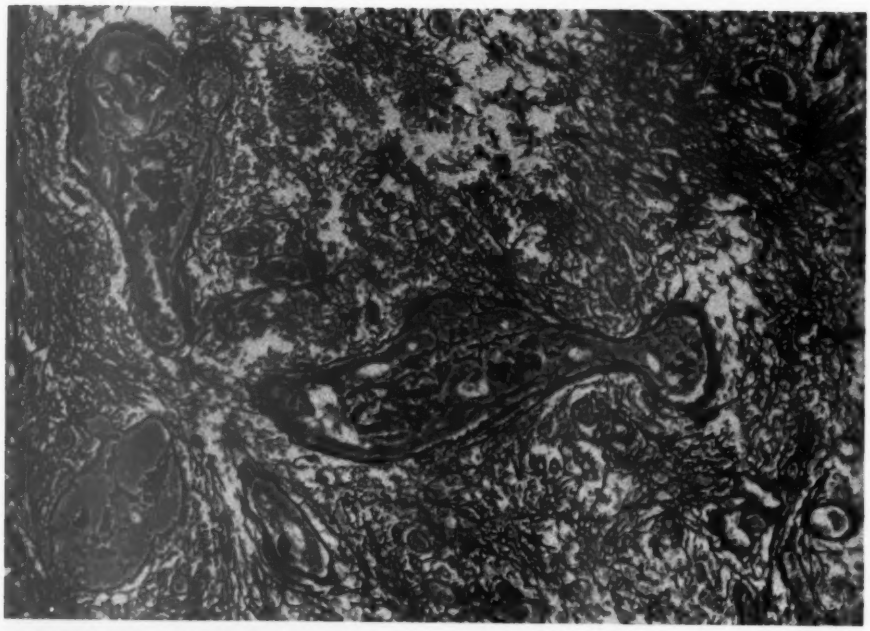


15

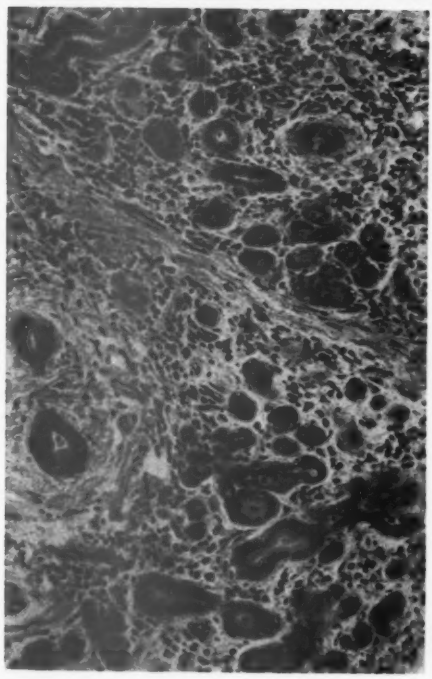
16

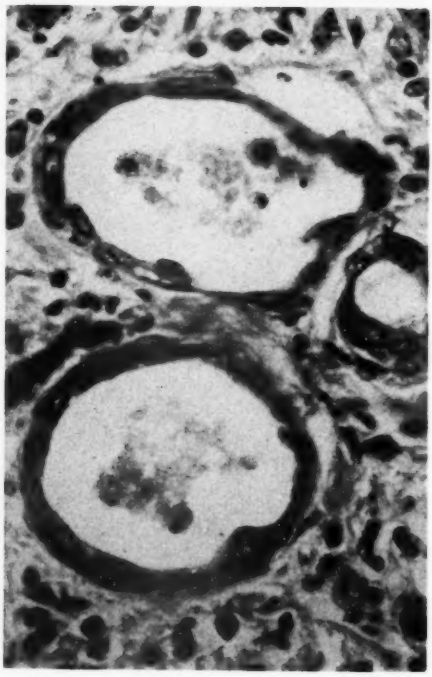
17

16



17



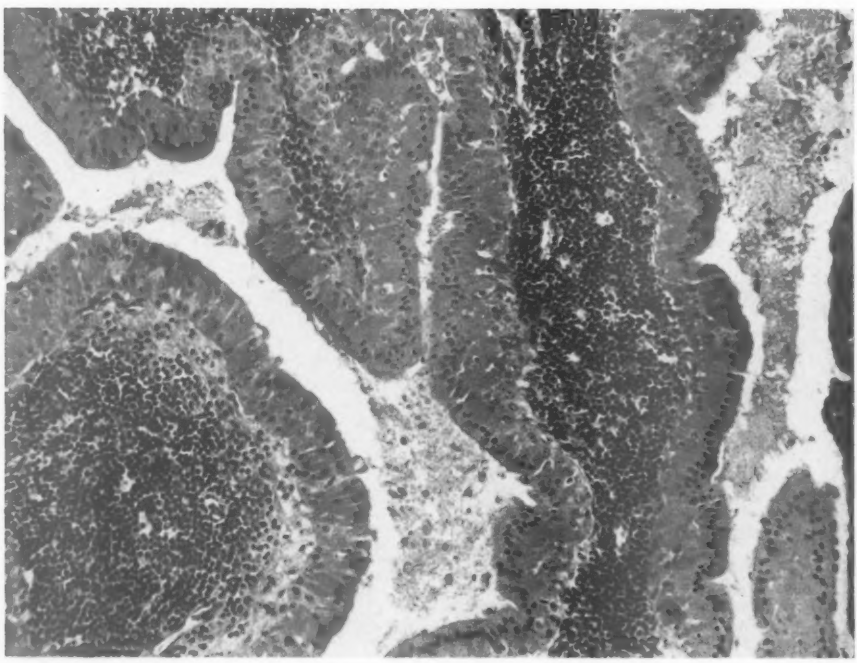


18

FIG. 19. Papillary adenocystoma lymphomatosum (Warthin's tumor). × 180.

FIG. 20. Malignant lymphoma of the salivary gland. Normal appearing ducts are present, epi-myoepithelial islands are absent. × 180.

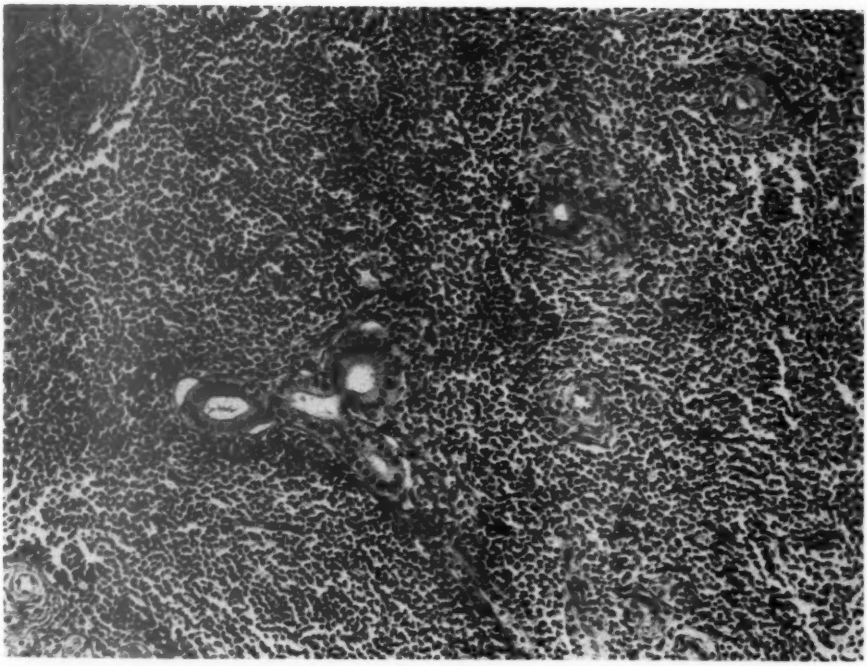
FIG. 21. Malignant lymphoma of the salivary gland. Another example showing atrophy of the ducts but no epi-myoepithelial islands. × 180.



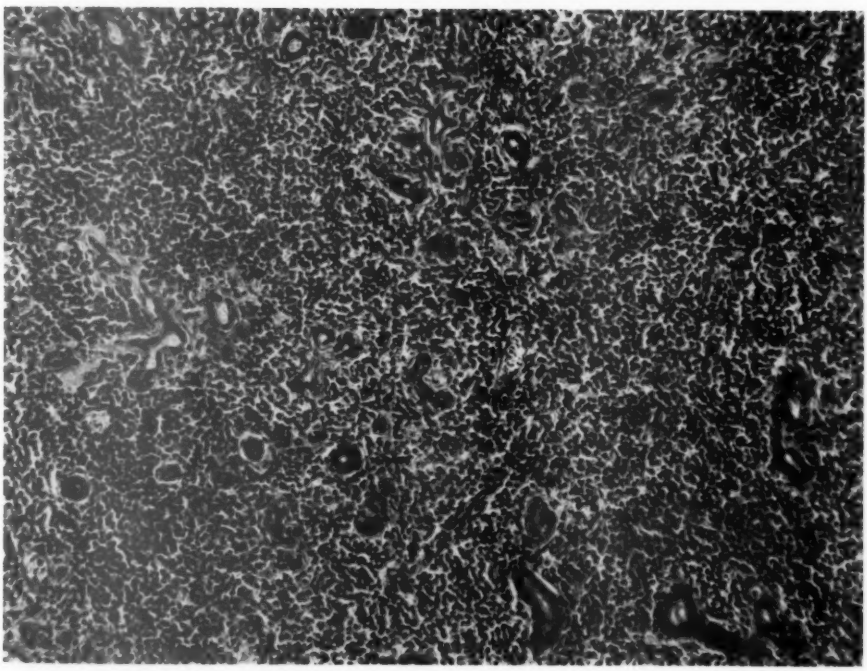
19

20

21



20



21

LYMPH NODE STRUCTURE IN PATIENTS WITH CANCER OF THE BREAST *

MAURICE M. BLACK, M.D., STASE KERPE, M.D., and FRANCIS D. SPEER, M.D.
(From the Department of Pathology, New York Medical College, Flower and Fifth Avenue Hospitals, New York 29, N.Y.)

This paper is an initial report of a systematic study of the lymph nodes in cancer patients. Particular attention has been directed to the axillary lymph nodes removed during radical mastectomy for carcinoma of the breast. An attempt has been made to correlate the microscopic structure of the node with the presence or absence of metastases, survival, and age of the patient.

This investigation was prompted by a chance observation made on CFW mice bearing spontaneous mammary carcinomas. We had the opportunity to observe the rare occurrence of spontaneous regression of the tumors in two such mice. In both cases the animals were sacrificed and a unique finding was obtained at necropsy, *viz.*, a replacement of the splenic follicular and sinusoidal architecture with sheets of large monocytoid cells. No such appearance was found in more than 150 spleens removed from other tumor-bearing mice of this strain; nor was any other instance of spontaneous tumor regression found in over 100 CFW tumor mice living out their life span. In view of these unusual findings it appeared worth while to undertake a study of the lymph nodes in human cancer cases to determine whether any association could be found between the microscopic appearance of the nodes and the biologic behavior of the tumor.

Our findings would indicate that the lymph nodes in an appreciable percentage of breast cancer patients show marked sinusoidal and follicular histiocytic transformation as well as amyloid-like changes. Such changes are most prominent in the nodes of patients known to have had 5-year cures, and a direct correlation was found between the occurrence of such changes in the nodes and the survival of individual cases. On the other hand, no relationship was observed between the appearance of the lymph nodes and the age of the patient, or between the structure of the lymph nodes and the histopathologic type of the primary tumor.

MATERIALS AND METHODS

In the present series the lymph nodes from 226 cases of breast carcinoma were examined. Of this group, 120 showed metastatic in-

* Aided by grants from the Leukemia Research Foundation and the Helen Andreadis Foundation.
Received for publication, November 13, 1952.

volvement of the lymph nodes (class II). The cancer tissue and lymph nodes studied were obtained largely from the surgical pathology files of the Department of Pathology of the New York Medical College Flower and Fifth Avenue Hospital. The sections had been prepared by the routine methods of formalin fixation and hematoxylin and eosin staining.

The lymph nodes were examined for changes in the sinusoids, follicles, and pulp. The microscopic appearance was evaluated in respect to the degrees of prominence of sinus histiocytosis and secondary follicles, and the character of the pulp. Some lymph nodes were found to show minimal microscopic differentiation, consisting essentially of a lymphocytic pulp without evidence of follicle formation or sinusoids (Fig. 1). Such nodes have been designated as having a zero rating for sinus histiocytosis and follicles. They were also negative in regard to amyloid-like changes or reticulo-endotheliosis of the pulp. With such an appearance as a reference, we have graded the intensity of sinus histiocytosis and of follicular hyperplasia from 0 to 4 plus. While most of the nodes from the same case tended to present a similar appearance, some variability was encountered. In such instances the case was rated according to the findings in the lymph node showing the most pronounced changes.

Sinusoids

A 4-plus reaction was characterized by prominent sinusoids throughout the node, with the sinusoids filled by large cells arranged in a compact, cord-like fashion and having eosinophilic cytoplasm and vesicular nuclei. Many of the nuclei showed a definite nucleolus (Figs. 2 and 3). In some nodes this reaction appeared to have its extreme expression in a sarcoid-like picture or in amyloid-like eosinophilic homogeneous change in the stroma. This appearance is to be differentiated from that occasionally seen in lymphadenitis in which dilated sinusoids filled with cells may be observed. In the latter instance, the cells show lipid histiocytosis, erythrophagocytosis, and also pleomorphism. Further, the peripheral sinus histiocytosis, common in the true 4-plus reaction, was lacking.

Follicles

A 4-plus reaction was characterized by diffuse prominence of hyperplastic follicles with secondary centers. The secondary centers were of two main types:

(a) The follicle consisted largely of a secondary center which tended to be monomorphic and was composed of a compact arrange-

ment of large monocytoid cells which had prominent nuclei and a well defined eosinophilic cytoplasm. The cytoplasmic borders tended to fuse so that a decided eosinophilic matrix was observed. In many ways the appearance of these cells was similar to that of the sinusoidal histiocytes. In some cases this central cellular proliferation was so marked as to result in fusion of neighboring follicles. Small blood vessels were usually a component of such centers. Secondary centers of this type, composed mainly of histiocytes and fibro-endothelial cells, were designated by Gillman and Gillman[1] as reactive in character. We shall therefore refer to them as R type follicles (Fig. 4). This appearance was in contrast to that of the second type of follicular reaction.

(b) In the second type, the follicles were characterized also by diffuse hyperplasia and large secondary centers. However, the secondary centers were composed of loosely arranged pleomorphic aggregates of cells. The cells included lymphocytes and lymphoblasts as well as large cells with ovoid or irregular nuclei having a delicate chromatin pattern. The cytoplasm of the various cells was sparse so that no eosinophilic ground work was observed. The appearance was similar to that of foliicles described by Gillman and Gillman[1] as hemohistioblastic and designated as germinal centers (Fig. 5). Such follicles will thus be referred to as G follicles. In some instances both types of reaction were observed in the same node.

Lymph nodes showing a pronounced degree of sinus histiocytosis, follicular hyperplasia, and reticulo-endotheliosis of the pulp presented a picture which we believe is best characterized by the term reactive.

Results

Sinus histiocytosis

Consecutive Series

Examination of the microscopic structure of the axillary lymph nodes in the series of consecutive cases of breast carcinoma revealed a decided variability in the degree of sinus histiocytosis, follicular hyperplasia, incidence of amyloid changes, and reticulo-endotheliosis of the pulp. In Table I we have listed the percentage incidence of the degrees of sinus histiocytosis of the lymph nodes encountered in the present series of 226 cases of operable breast carcinoma. It should be noted that there is no evidence of a single most frequent value for sinus histiocytosis as would obtain in the measurement of a parameter of a homogeneous population, *i.e.*, a characteristic bell-shaped curve. Instead we find a varying incidence for the different degrees of sinus histiocytosis, a finding more consistent with measurements made on a

heterogeneous population. Under such circumstances an arithmetic mean value is not adequately representative of the behavior of the group. However, it should be realized that clinical reports of results in terms of percentage of 5-year survivals of operable breast carcinoma are analogous to mean values and also give no indication of heterogeneity. For this reason and with the aforementioned limitation in mind, we have calculated the mean value for the degree of sinus histiocytosis in this consecutive series. The mean value was found to be 1.97 plus.

In order to evaluate the significance of the findings in the consecutive series, we have attempted to obtain follow-up information on the

TABLE I

Varying Degrees of Sinus Histiocytosis of the Axillary Lymph Nodes in a Consecutive Series of Cases of Breast Cancer

Series	No. of cases	Degree of sinus histiocytosis					χ^2	P*
		0	1	2	3	4		
Consecutive	226	31%	15%	13%	18%	23%		
Class II	120	51%	12%	11%	12%	13%	23.4	<0.001

* Probability that such a value of χ^2, calculated on the basis of 4 degrees of freedom, would be due to chance occurrence.

individual cases. To date such information has been obtained in 91 cases. These data have been studied in order to clarify what relationship, if any, exists between the microscopic appearance of the axillary lymph nodes and the behavior of individual cases. In Table II we have indicated the varying degrees of sinus histiocytosis encountered in the series of follow-up cases. These values as well as those of the consecutive series have been used as reference curves against which to compare the selected series which follow.

Patients with Five-Year Cures

To date we have examined the lymph nodes from 53 patients known to have had 5-year cures, *i.e.*, apparently free of disease for 5 years postoperatively. In Table II we have indicated the percentage incidence of the varying degree of sinus histiocytosis in this series. As compared with the consecutive series of cases or the total follow-up series, there is a decreased incidence of the sinus histiocytosis graded zero, and an increase in the higher values. These changes appear to be statistically significant as judged by the χ^2 value obtained when comparison is made with either the consecutive series or the total follow-up series. It should also be mentioned that the mean value is somewhat higher than that of the consecutive series, being 2.60.

Patients Dying of Breast Cancer in Less Than Three Years

A more striking contrast is provided when one makes a similar comparison with those patients known to have died of cancer of the breast in less than 3 years. The incidence of the various degrees of sinus histiocytosis found in this group of cases surviving less than 3 years is indicated in Table II. The most striking feature of this group, in contrast to the series of 5-year cures, is that 79 per cent of the cases had sinus values of less than 2 plus. The distribution of values obtained in this selected group is significantly different from the consecutive series or the total follow-up group as judged by the χ^2 method of testing. Of equal importance is the *absence* of any cases with sinus values of 3

TABLE II

Varying Degrees of Sinus Histiocytosis of Axillary Lymph Nodes in Cases with Followed Records

Series	No. of cases	Degree of sinus histiocytosis					χ^2	P*
		0	1	2	3	4		
Total	91	28%	13%	21%	20%	18%		
5-year cure	53	13%	9%	21%	26%	30%	9.57	0.05
Dead in 3 years	19	58%	21%	21%			14.25	0.01

* Probability that such a value of χ^2, calculated on the basis of 4 degrees of freedom, would be due to chance occurrence.

plus or more. The mean value of the degree of sinus histiocytosis in this group of short-term survivors was 0.66.

The absence of lymph node sinus readings greater than 2 plus in the group of cases with short-term survival and the finding that the mean sinus value for the cases with 5-year cure was 2.6 plus suggested that cases having sinus histiocytosis values of 3 plus or more might be assured of a 5-year cure in almost every case. In this connection, let us once again consider the percentage incidence of the various degrees of sinus histiocytosis found in the consecutive series. As will be seen in Table I, 41 per cent of these cases showed sinus histiocytosis readings of 3 plus or more. From the above considerations we would then expect that the *minimal 5-year survival* of an unselected series of operable cases of breast cancer would be 41 per cent. This value is in excellent agreement with clinical findings.

Comments on Sinus Histiocytosis

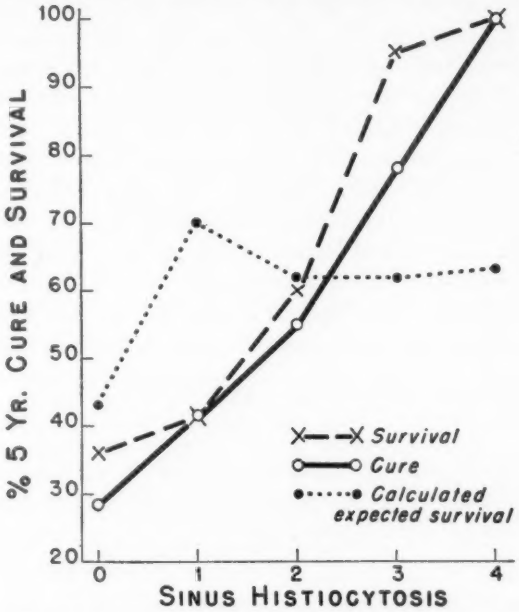
From the foregoing data it appears that cancer of the breast is associated with varying degrees of sinus histiocytosis of the axillary nodes. There also seems to be a relationship between the intensity of sinus

histiocytosis and survival. It should be noted that in presenting the foregoing data, we have not attempted to make any separation into class I, without axillary metastases, or class II, with axillary metastases. At this point, however, it would be well to consider this aspect of the problem. We are well aware of the fact that a certain number of class I cases would be more properly classified as class II had serial sections been made on all nodes available. However, it is self evident that in those cases in which metastases to the lymph nodes have been found there is no possibility of their being class I cases. One may thus compare the findings in the class II group with the entire series of consecutive or follow-up cases. In Table I we have included the varying degree of sinus histiocytosis found in this group of class II cases. As judged by the χ^2 value, the distribution of sinus histiocytosis in this group is significantly different from that of the consecutive series as a whole. A very striking finding is that 25 per cent of the cases of this series show sinus histiocytosis of 3 plus or greater. In line with our previous discussion of the relationship between survival and sinus histiocytosis, it might be inferred that the minimal 5-year survival expected in cases of operable breast carcinoma with axillary metastases would be 25 per cent. As a minimal prediction, this value is not inconsistent with the clinical results reported in the literature. Attention should also be called to the finding that the mean value for sinus histiocytosis in this group is 1.32 plus, a value less than that for the consecutive series as a whole or the 5-year cure group, but higher than that obtained for the series of patients dying in less than 3 years. Thus, in the four selected groups of cases indicated in Tables I and II, there is a parallel relationship between the mean value for sinus histiocytosis and survival.

When the followed cases are considered in terms of the number of cases showing axillary metastases, a striking observation is made, *i.e.*, when sinus histiocytosis is 3 plus or more there is no significant difference between the survival of those cases known to have axillary metastases and those presumed to be free of axillary metastases or the group as a whole. In Text-figure 1 we have plotted the percentage of 5-year cures and 5-year survivals (living with evidence of disease) against the degree of sinus histiocytosis. In addition, we have indicated the expected 5-year survival based on the number of class I and class II cases, assuming an expected 5-year survival of 80 per cent for class I cases and 40 per cent for class II cases. Thus of 16 cases showing 4 plus sinus histiocytosis, 7 were known to have had axillary metastases while in 9 no metastases were found. The expected number

of survivals would be: 9 class I cases × 0.80, plus 7 class I cases × 0.40, or 10 cases. The expected percentage of survival would thus be 10/16 × 100 or 62.5 per cent.

It is readily apparent from Figure 1 that there is a linear relationship between the percentage of survival and the degree of sinus histiocytosis. This relationship is independent of the presence or absence of axillary metastases. It should be mentioned also that 32 of 33



Text-fig. 1. Relationship between the degree of sinus histiocytosis of axillary lymph nodes and 5-year survival and cure. Of note is the lack of prognostic significance of the survivals as calculated on the basis of presence or absence of lymph node metastases.

patients whose lymph nodes showed sinus histiocytosis of 3 or 4 plus were alive at the end of 5 years. In this group, 15 of the cases proved to have axillary metastases. Statistic comparison of such a survival with the expected calculated survival, as previously indicated, reveals that the probability is less than 1 in 1,000 that this could be due to a chance occurrence.

APPEARANCE OF FOLLICLES

Certain generalizations may be made in regard to the appearance of the nodal follicles in the cases of breast cancer. There appeared to be a tendency for the reactive type of follicular hyperplasia to be associated with significant degrees of sinus histiocytosis and to occur

more frequently in cases showing superior survival. However, in the lymph nodes of cases of breast cancer, this feature was not nearly so striking as sinus histiocytosis. Additional studies will be required to clarify the relative significance of reactive follicles and of sinus histiocytosis in defining the activity of lymph nodes as related to survival.

Amyloid-like Changes and Reticulo-endotheliosis of the Pulp

While amyloid-like changes and reticulo-endotheliosis of the pulp are not readily expressed by numerical values, certain general impressions have been obtained in the present study. There appears to be a relationship between the incidence and intensity of amyloid-like changes, of reticulo-endotheliosis of the pulp, and of the degree of sinus histiocytosis, and survival. We have a subjective impression that marked sinus histiocytosis, follicular hyperplasia of reactive type, amyloid-like changes, and reticulo-endotheliosis of the pulp all seem to be component structural indications of a process which for want of a better term we have called nodal reactivity. Of utmost significance in the present study is the observation that the reactive appearance of the lymph node is associated with excellent clinical survivals.

LYMPH NODE STRUCTURE IN CASES OF CANCER OTHER THAN OF THE BREAST

We have not as yet accumulated equivalent data; however, preliminary work indicates that variations in nodal appearance occur also in cancer cases of other types. That there may be similar prognostic significance is suggested by the findings in the following cases:

B. B., a 35-year-old male, developed a teratoma of the left testicle in 1943. Positive Aschheim-Zondek tests were obtained on several occasions. Unilateral orchiectomy was performed and he was well until 1947 at which time a mass was noted on the right side of the neck lateral to the thyroid gland. Biopsy revealed this mass to be a metastatic papillary adenocarcinoma of the thyroid gland in a lymph node. Thyroidectomy and right-sided neck dissection were performed. Examination of the uninvolved lymph nodes removed at this time revealed 4 plus sinus histiocytosis and amyloid-like changes. The patient has been well and apparently free of disease to the present (November, 1952).

M. D., a 16-year-old female, developed a melanoma of the right breast. A radical mastectomy was performed and the axillary nodes were found to be involved with metastases. The uninvolved nodes showed dilated sinusoids, but sinus histiocytosis was minimal (1 plus). Neither follicular hyperplasia or amyloid-like changes were noted. The disease progressed rapidly and she expired from cerebral and pulmonary metastases within 18 months of the operation.

M. H., a 38-year-old female, developed melanoma of the scalp in October, 1946. Dissection of the regional nodes revealed metastatic involvement. The uninvolved nodes were devoid of sinus histiocytosis or follicular hyperplasia. She expired from extensive local and general dissemination in 3 months.

M. F., a 20-year-old female, developed a melanoma of the right thigh in 1949. A wide local excision with dissection in continuity of the inguinal lymph nodes was carried out. No evidence of tumor metastases was noted. Microscopic examination of the lymph node revealed marked sinus histiocytosis at that time. She was well until the early part of 1952 when a right inguinal mass was noted. Exploration revealed metastatic melanoma in the regional nodes but not in the higher nodes along the iliac veins and aorta, which also were dissected. The uninvolved nodes again showed a marked sinus histiocytosis (4 plus). To date the patient is well and shows no evidence of further disease at this time (November, 1952).

A. D., a 26-year-old male, developed a beltline melanoma of the back in February, 1952. A dissection in continuity of the left side and the removal of the lymph nodes of the inguinal region and retroperitoneum along the aorta were carried out. No evidence of metastases was found, but all of the lymph nodes showed an extreme degree of sinus histiocytosis and amyloid-like changes. It is too early to judge long-term survival, but as far as can be determined he is free of disease at this time (November, 1952).

The last two cases are of interest also because nodes at some distance from the original tumor and nodes other than those of the axillae were available for study. The findings therein are consistent with a systemic and widespread change in the lymph nodes rather than a local alteration in response to some type of local irritation or chemical product of the tumor.

LYMPH NODE STRUCTURE IN NON-CANCER PATIENTS

It should be pointed out at this time that we are not inferring that sinus histiocytosis, R type follicular hyperplasia, or amyloid-like changes do not occur in patients free of carcinoma, or, for that matter, in patients free of any apparent disease. Gillman and Gillman[1] have described such histologic characteristics as part of the variable lymph node picture presented in normal rats. In an attempt to determine to what extent the reactive lymph node is found in individuals without cancer, we have initiated a study of the lymph nodes from necropsy material and, as available, from surgical material for biopsy from non-cancer patients of various ages. In 30 such cases examined thus far, only 2 instances of the reactive lymph node picture have been found. Additional studies are in progress on the lymph nodes of non-cancer patients and also on lymph nodes obtained by necropsy from cancer patients. It should be noted, however, that other observers have commented upon the lymphoid hyperplasia occurring in human cancer patients and in experimental cancerous animals.[2-4] Thus Bateman, Klopp, and Mendelson[2] stated "That growing tumor is associated with lymph node hyperplasia, even in the absence of metastatic involvement, was particularly apparent in the cases of carcinoma of the breast observed in this study. Only a few small axillary nodes were obtainable from noncancer patients. In the presence of malignant neoplasm

of the breast, large nodes were found in which no neoplastic cells were seen microscopically, and such cancer-free nodes greatly outnumbered those in which metastatic cancer was found."

Histology of the Primary Tumor. In the present study almost all histologic varieties of breast carcinoma were observed, including mucogenic, scirrhous, anaplastic, adenocarcinoma, comedo, and medullary types. It has not been possible thus far to correlate the microscopic appearance of the primary tumor, the presence or absence of necrosis therein, or the size of the primary tumor and its presumed duration before resection, with the appearance of the regional lymph nodes.

Age of the Patient. In view of the known alteration of lymphoid tissue with advancing age, it was important to evaluate the possible influence of this variable on the structure of lymph nodes. However, it soon became apparent that no such correlation could be made in the large consecutive series or in the followed cases. In the latter, the mean age and distribution of ages were essentially the same in the groups with different degrees of sinus histiocytosis. The mean ages of the different groups were as follows: sinus hyperplasia, 0, 50 years; 1 plus, 54 years; 2 plus, 51 years; 3 plus, 52 years; 4 plus, 52 years.

Discussion

The major interest in the lymph nodes of cancer patients has been in regard to the presence or absence of metastatic involvement and the prognostic significance thereof. The presence or absence of lymph node metastases is generally accepted as the most valuable prognostic sign in operable breast carcinoma. However, despite its real prognostic value, the mere statement of the presence or absence of metastases in the lymph nodes fails to account for the 20 per cent of deaths in the class I cases after 5 years and the 60 per cent of deaths after 10 years, or the 40 per cent of 5-year survivals and 17 per cent of 10-year survivals in the class II cases. Further, it is not even applicable in understanding the occurrence of long survivals in cases of inoperable breast cancer. It is just such variability in behavior within supposedly homogeneous groups which has led to the concept of "biological predeterminism"[5] in cancer and has seriously challenged the current concept of "early diagnosis" and radical surgical procedures as valid instruments for the control of cancer.[6,7] In short, there is strong indication that the clinical history of cancer patients is determined by variable factors residing in the tumor, or in the host, or in both.[8] However, to our knowledge, we are still lacking in the demonstration of any parameter of measurement which would express the controlling influence of the host on the tumor.

It is therefore pertinent to examine the results of the present investigation in the perspective of this discussion. The present data indicate that there is excellent parallelism between the relative incidence of cases with marked sinus histiocytosis, or the mean values for the sinus histiocytosis of lymph nodes, and the periods of survival of the various series studied. To this extent the microscopic appearance of the nodes might be a reflection of the presence or absence of metastasis to the node. However, a more fundamental significance is suggested when we consider the data depicted in Text-figure 1. These indicate that the presence or absence of lymph node metastases is less well correlated with survival than is the microscopic appearance of the lymph nodes. It would appear that the poor survival of class II cases is related not so much to the presence of lymph node metastases, as to the fact that cases of this type show a smaller incidence of sinus histiocytosis of 3 and 4 plus grades.

The finding of sinus histiocytosis graded 3 plus or more in the axillary nodes of patients with breast cancer is almost invariably associated with a survival of 5 years or more regardless of the presence of axillary metastases. The universality of this association between survival and the microscopic appearance of the lymph nodes must perforce overshadow any other variables, *viz.*, biochemical variability of the primary tumor, presence or absence of axillary metastases, or therapeutic intervention. However, it should be noted that while cases with lesser degrees of reactivity of the lymph nodes show diminishing survival rates, some patients do experience 5-year cures despite a complete lack of any reactivity of the sinuses, follicles or pulp. Such findings would be consistent with a hypothesis that two major variables are operable in determining the survival of patients with breast cancer, *viz.* (a) a factor related to the host, made evident, at least in part, by the microscopic appearance of the lymph nodes, and (b) the variables associated with the primary tumor which would include the biochemical characteristics of the primary tumor, surgical cures, and palliative therapy.

The recent literature contains reports on the occurrence of granulomatous sarcoid-like changes in the regional lymph nodes of cancer cases.[9,10] While we have observed such changes in the nodes which we have examined, they do not seem to occur with any regularity nor can they be correlated with class or behavior of the individual cases. In some instances they appear to be associated with an extreme expression of sinus histiocytosis, while in others they are unrelated to any other histologic feature of the lymph nodes. However, it is pertinent to note that in a paper by Symmers[9] on "Granulomas Associated with

Carcinoma," he did mention the occurrence of sinus histiocytosis and amyloid-like changes in the nodes of some of his cases. Interestingly enough, his case reports indicate excellent therapeutic results in these cases.

Despite the very evident need for further work in this field, it appears justified to include the microscopic appearance of the axillary lymph nodes among the variables, determining or prognosticating the clinical course of patients with breast cancer. This variable appears to indicate the clinical course more closely than the histologic appearance of the tumor, the age of the patient, the presence or absence of axillary metastases, or the stated duration of the tumor before surgical resection.

The present findings are also of interest in regard to the unproved but persistent and recurrent feeling that the reticulo-endothelial system is concerned in some way with the control of tumor growth.[11] It is readily apparent from our studies as well as from those of Gillman and Gillman[1] that lymph nodes may present marked functional and morphologic variability. It therefore follows that the use of splenic or lymph node extracts without determination of the microscopic architecture of the source material will yield a heterogeneous and highly varied material. The chemical variations in lymph nodes of different microscopic appearance are still unknown and are under investigation in our laboratory.

It would appear that sinus histiocytosis, monocytoid hyperplasia of the follicles, and amyloid-like changes reflect alteration in a common target structure—endothelium. In this connection it may be pertinent to recall that in a previous study of tumor dehydrogenase activity we[12] showed that the tumor stroma and particularly the endothelial component thereof, undergoes unique metabolic activity in many human tumors and uniformly in many varieties of mouse mammary carcinoma. In an effort to ascertain the relationship, if any, between such findings in the primary tumor and the appearance of the lymph nodes, we have undertaken a study of the dehydrogenase activity of the primary tumor and the lymph nodes. These findings will be reported at another time. Continued work is in progress to evaluate further the conclusions drawn from the findings to date and to assess their general applicability in diverse types of cancer. Additional reports will be forthcoming as such data have been evaluated.

Summary

An attempt has been made to correlate the microscopic appearance of the lymph nodes in cancer patients with the presence or absence of

metastases, survival, age of the patient, and the appearance of the primary tumor. Particular reference has been paid to cases of breast carcinoma. Our findings indicate that the lymph nodes in an appreciable percentage of patients with breast cancer show marked sinusoidal and follicular histiocytic transformation as well as amyloid-like changes. Such changes are more prominent in cases with longer survival. A direct correlation was found also between the occurrence of such changes in the nodes and the survival of individual cases. On the other hand, no relationship was observed between the lymph node appearance and the age of the patient, or the histopathologic type of the primary tumor. Similar findings have been observed also in other types of cancer. Extension of these studies is in progress.

REFERENCES

1. Gillman, J., and Gillman, T. The pathogenesis of experimentally produced lymphomata in rats (including Hodgkin's-like sarcoma). *Cancer,* 1952, **5,** 792–846.
2. Bateman, J. C., Klopp, C. T., and Mendelson, A. A comparison of nitrogen content of lymph nodes from cancer and noncancer patients. *Cancer Research,* 1951, **11,** 777–779.
3. Sampson, J. A. A careful study of the parametrium in twenty-seven cases of carcinoma cervicis uteri and its clinical significance. *Am. J. Obst. & Gynec.,* 1906, **54,** 433–464.
4. Homburger, F. Studies on hypoproteinemia: III. Lymphoid hyperplasia and redistribution of nitrogen caused in mice by transplanted tumors (sarcoma 180 and breast adenocarcinoma EO 771). *Science,* 1948, **107,** 648–649.
5. Macdonald, I. Biological predeterminism in human cancer. *Surg., Gynec. & Obst.,* 1951, **92,** 443–452.
6. McKinnon, N. E. Cancer of the breast. The invalid evidence for faith in early treatment. *Canad. J. Pub. Health,* 1951, **42,** 218–223.
7. Park, W. W., and Lees, J. C. The absolute curability of cancer of the breast. *Surg., Gynec. & Obst.,* 1951, **93,** 129–152.
8. Black, M. M., Bolker, H., and Kleiner, I. S. Some considerations of morphology and metabolism in malignant neoplasia. *New York State J. Med.,* 1950, **50,** 309–315.
9. Symmers, W. St. C. Localized tuberculoid granulomas associated with carcinoma. Their relationship to sarcoidosis. *Am. J. Path.,* 1951, **27,** 493–521.
10. Nadel, E. M., and Ackerman, L. V. Lesions resembling Boeck's sarcoid in lymph nodes draining an area containing a malignant neoplasm. *Am. J. Clin. Path.,* 1950, **20,** 952–957.
11. Stern, K., and Willheim, R. The Biochemistry of Malignant Tumors. Reference Press, Brooklyn, New York, 1943, pp. 714–745.
12. Black, M. M., Kleiner, I. S., and Speer, F. D. Vascular participation in tumor slice metabolism. *Proc. Soc. Exper. Biol. & Med.,* 1951, **77,** 206–208.

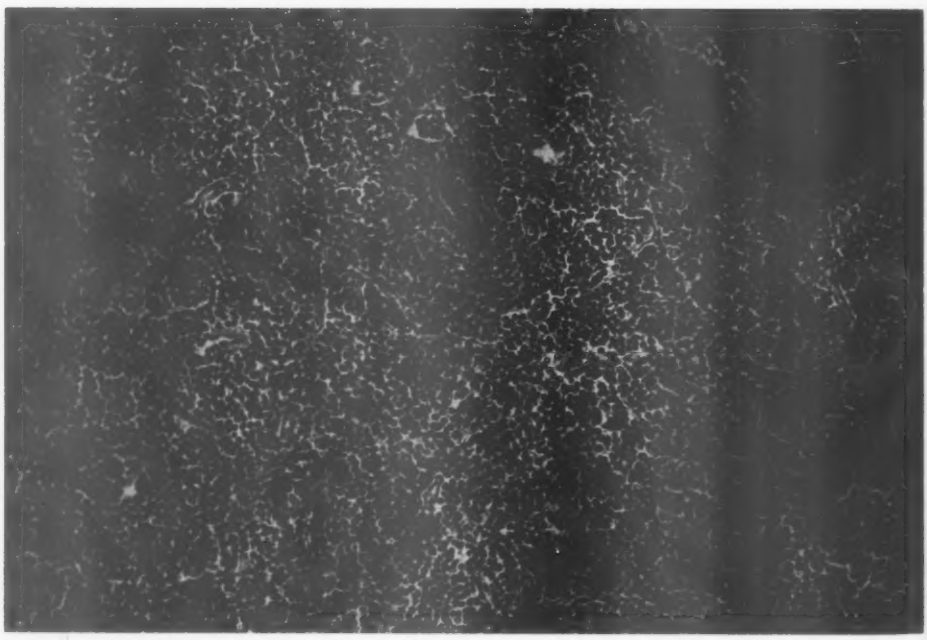
[*Illustrations follow*]

LEGENDS FOR FIGURES

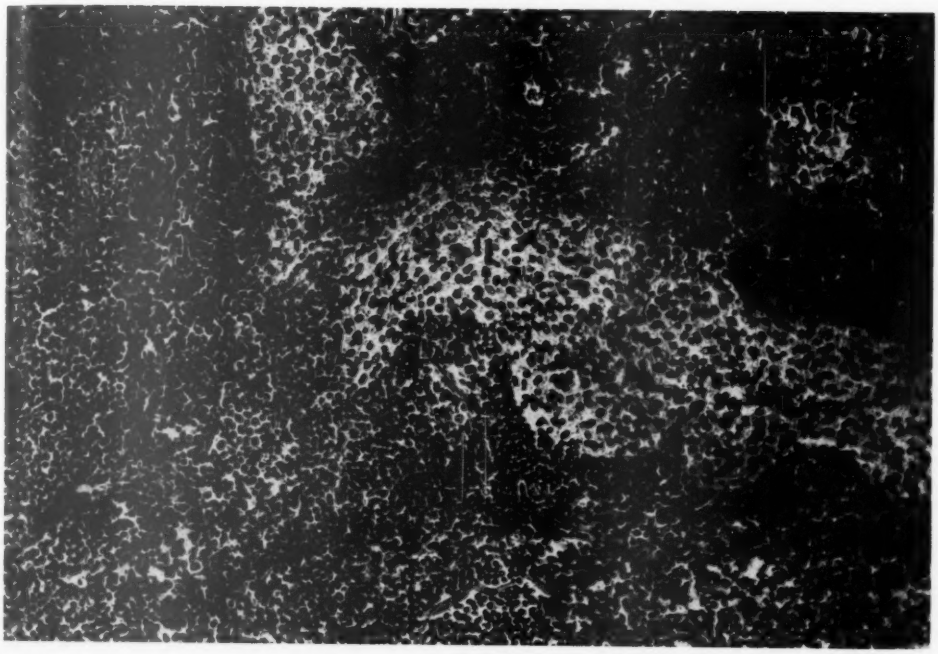
FIG. 1. Lymph node showing diffuse lymphocytic proliferation without follicular centers or sinusoidal prominence. This appearance is the prototype for a sinusoidal and follicular rating of zero.

FIG. 2. Sinus histiocytosis, 4 plus. There are dilated sinusoids filled with solid cords of proliferated histiocytes.

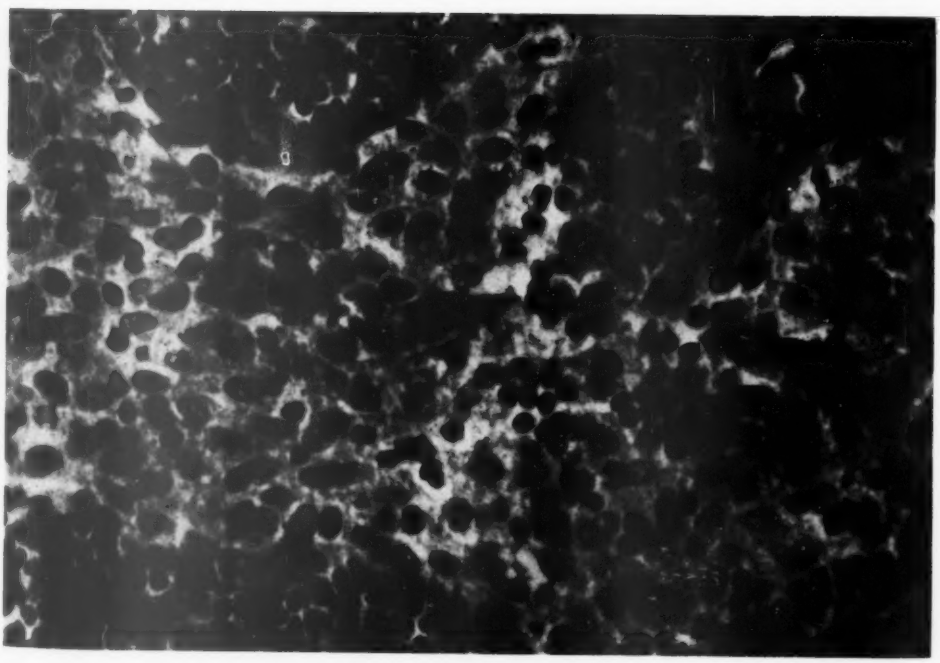
FIG. 3. High-power view of sinus histiocytosis. The cytoplasm and nucleoli of the histiocytes are prominent.



1



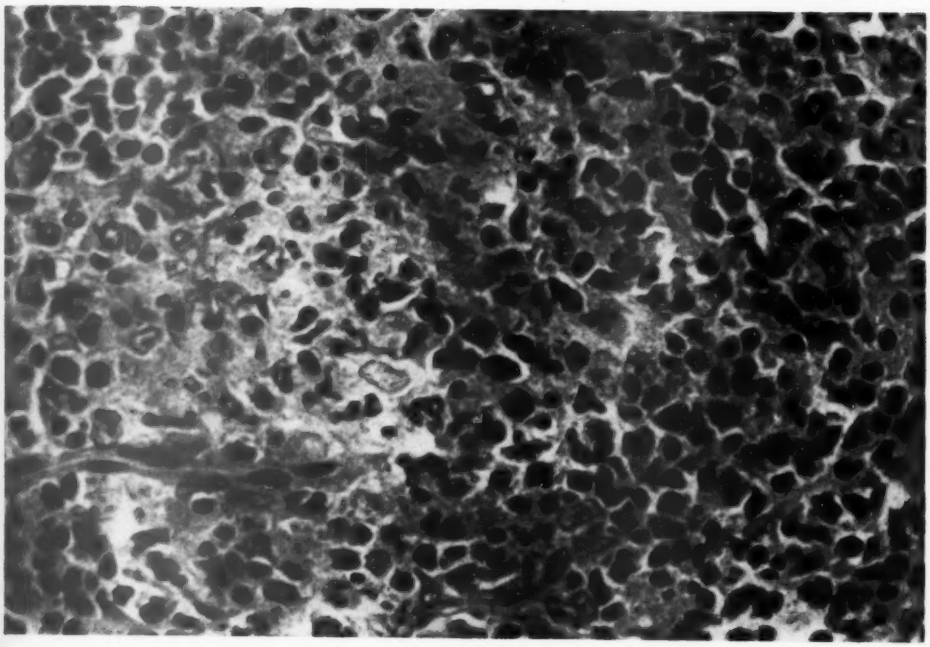
2



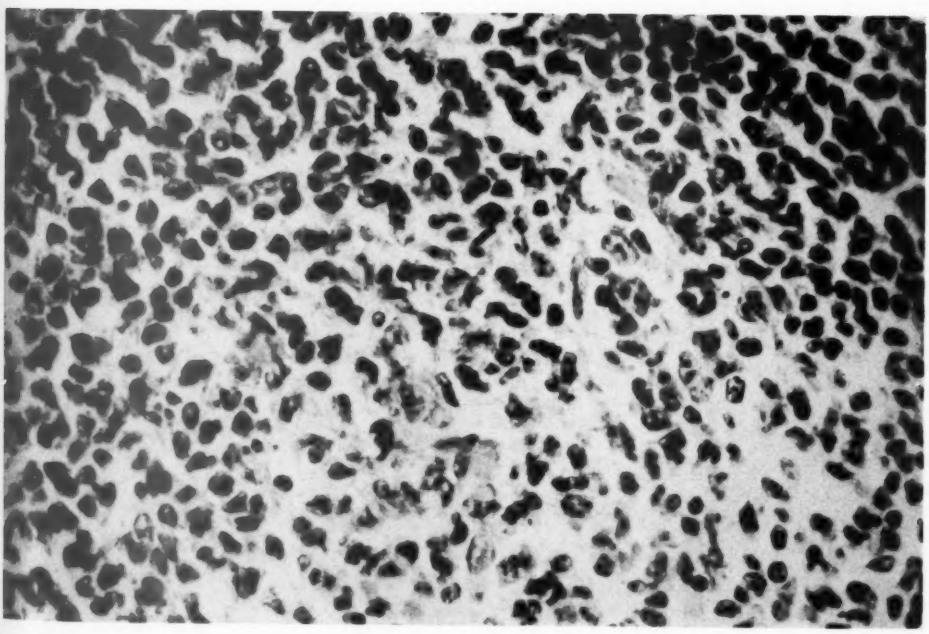
3

FIG. 4. Histiocytic and fibro-endothelial cell proliferation in secondary center, typical of reactive (R) follicle. The small vessels are prominent and there is a definite background matrix.

FIG. 5. Germinal center (G) type of follicular reaction. The pleomorphism and the loose arrangement of cells with minimal cytoplasm are readily apparent.



4



5

A HISTOCHEMICAL STUDY OF THE DISTRIBUTION AND FATE OF DEXTRAN IN TISSUES OF THE MOUSE *

ROBERT W. MOWRY, M.D.,† and R. CARL MILLICAN, M.D.

(From the Laboratory of Pathology and Pharmacology, National Institute of Arthritis and Metabolic Diseases, the National Institutes of Health, Public Health Service, Federal Security Agency, Bethesda 14, Md.)

Dextran‡ is a promising plasma substitute.[1-3] While it is probably antigenic, no major toxicity has been reported as yet from its use.[4] Since patients in shock may require large amounts of dextran, much attention has been given to the excretion of this material by the body. It is established that about one third of all injected dextran is eliminated in the urine, largely during the first 24 hours.[2,5,6] The fate of the remaining dextran is not clear. It has been said that dextran should not be regarded as safe for general use until the possibility of long-term storage by the body is excluded.[3]

Plasma levels of dextran decline rapidly and reach zero a week or so after injection. Even after very large total doses of dextran, no chemical or histologic evidence of storage has been found.[2,3,7-9] Thus, some workers believe that storage does not occur and that dextran is metabolized. Chemical methods that have been used for the assay of dextran in tissues are not as reliable as those used for measuring dextran in blood and urine. Some direct evidence that dextran may be partly metabolized, at least, has been obtained only recently. Gray, Siiteri, and Pulaski[10] found an increased output of glucose in the urine of phloridzinized, fasting dogs given dextran as compared to controls not given dextran. Further, radioactive carbon dioxide was recovered from the expired air of both mice and dogs after the injection of C^{14}-labelled dextran.[11,12]

On the other hand, there is certain evidence against the view that dextran is always metabolized. No dextran was used up when known amounts were added to homogenates or extracts of various organs.[3,13,14] Engstrand and Åberg[13] found dextran in gastric juice but none in feces. From these findings they reasoned that much dextran may be excreted into the gastro-intestinal tract and later may be hydrolyzed by coliform bacteria. Foam cells have been noted in spleens of dextran-treated animals and regarded as evidence of stor-

* Presented at the Forty-ninth Annual Meeting of the American Association of Pathologists and Bacteriologists, New York City, April 10, 1952.

Received for publication, November 24, 1952.

† Now at Department of Pathology, Washington University School of Medicine, St. Louis 10, Mo.

‡ As used in the text, dextran refers to partly hydrolyzed or clinical dextran.

age.[15] Finally, Bull and his co-workers[8] detected dextran by serologic methods in lymph nodes and spleens of rabbits as long as 2 months after injection.

Previous histologic studies on dextran were limited by the failure to stain dextran in tissue sections. Methods permitting the coloration of dextran in tissue sections were only recently evolved.[16] Additional work by us has led to a very simple and dependable method for the histochemical study of dextran. This improved method has been applied to a study of the distribution and fate of intravenously injected dextran in the mouse. The histochemistry of dextran will be presented before describing the results of our experiments.

PART I: THE HISTOCHEMICAL STUDY OF DEXTRAN

Fixation and Cutting of Sections

As dextran is known to be water-soluble, tissues containing dextran must be kept away from water. Accordingly, thin blocks of tissue are fixed in cold absolute alcohol and maintained at −5° C. for at least 48 hours. Next, the cold alcohol is poured off quickly and replaced by unchilled alcohol. Tissues are now kept in alcohol at room temperature about 18 hours. After two baths of petroleum ether, 1 hour each, tissues are infiltrated with paraffin and embedded. Blocks are cut at 5 μ without allowing either block or sections to come into contact with water. Cut sections are floated on absolute alcohol and mounted on clean glass slides, using a minimum of egg-albumen well rubbed off. Sections are dried at room temperature and put into a paraffin oven (60° C.) for at least 2 hours. Afterwards, slides are quickly cooled. The last-named precautions minimize section losses during the subsequent staining procedures.

The Demonstration of Dextran in Tissue Sections

Dextran is a polysaccharide composed of branched chains of unsubstituted glucose units. This means that dextran contains numerous 1,2-glycol linkages. Periodic acid is known to oxidize 1,2-glycols to aldehydes.[17] By the use of Schiff's reagent, insoluble aldehydes can be colored red and thereby localized in tissue sections. This is the basis for the periodic acid-Schiff's reaction as used in histologic technique.[18,19] Thus, dextran should be deeply colored by Schiff's reagent after periodic acid oxidation. Spot tests with solutions of dextran show that this is true.

The periodic acid-Schiff's reaction is ordinarily performed with aqueous reagents. When tissue sections of dextran-treated animals are

stained with aqueous reagents, no dextran is found. Even if tissues are fixed and cut as prescribed in the preceding, no dextran is found after aqueous periodic acid. Dextran is dissolved by the water and lost from sections.

In the original method of Mowry, Longley, and Millican,[16] dextran was kept insoluble during staining by the use of periodic acid-Schiff's reagents made in 70 per cent alcohol instead of water. By their method, dextran was intensely colored. Independently, Friberg, Graf, and Åberg[20] reported the staining of dextran by the periodic acid-Schiff's reaction. From the published account of these workers, it is not clear what precautions were taken to prevent the loss of dextran by exposure to water.

Since the periodic acid-Schiff's reaction is not specific for dextran, additional evidence is required for the presumptive identification of dextran in sections. Polysaccharides other than dextran that are composed of unsubstituted glucose residues should be equally reactive. From the beginning, we have used two precautions in the study of dextran. First, sections stained for dextran are always compared with duplicate sections exposed to water, either before or during periodic acid-Schiff's staining. Dextran deposits are dissolved and therefore absent in water-treated sections. This distinguishes water-soluble from water-insoluble periodic acid-Schiff's positive materials. In our experience, glycogen is not appreciably dissolved by water and will usually be present in both sets of slides. Second, tissues of animals or individuals not given dextran are examined by the same methods used for tissues of dextran-treated animals. This determines whether or not there are any naturally occurring dextran-like substances in the various tissues under study. Such substances, if present, might otherwise be mistaken for dextran. The principles outlined should be applicable to the histochemical study of water-soluble but alcohol-insoluble periodic acid-Schiff's reactive substances other than dextran.

It has been shown recently[21] that periodic acid can be used for histochemical purposes in concentrations of alcohol higher than 70 per cent. Since the oxidation of dextran by periodic acid appears to proceed more slowly in higher levels of alcohol, a longer time is required for optimal demonstration. Further, we have observed that dextran becomes insoluble in water after oxidation by periodic acid in alcohol. Not all water-soluble materials are rendered insoluble by periodic acid in alcohol; for example, oxypolygelatin. The finding that oxidized dextran is no longer soluble in water permits the use of aqueous Schiff's reagent and a wide choice of counterstains. The new method

finally evolved demonstrates much more dextran than our earlier method. This is believed due to more complete periodic oxidation and to the use of aqueous Schiff's instead of the less sensitive alcoholic Schiff's reagent. Details of the latest method follow.

Alcoholic Periodic Acid-Aqueous Schiff's Method for Dextran

1. Pass sections through xylene and alcohol to 95% alcohol.
2. Treat sections for 2 hours in a freshly made 1% solution of periodic acid ($HIO_4 \cdot 2H_2O$) in 90% ethyl alcohol.
3. Rinse sections in 80% alcohol and take to water.
4. Immerse for 10 minutes in (aqueous) Schiff's reagent. We use Lillie's 1 gm. % leukofuchsin.[22]
5. Rinse in three changes of M/20 sodium metabisulfite ($Na_2S_2O_5$) for 1, 2, and 2 minutes. Rinses are freshly made from a stock molar solution (19%) of sodium metabisulfite.
6. Wash for 2 to 5 minutes in running tap water. Counterstain if desired, dehydrate, clear, and mount. (Dextran is colored deep purple, almost black. Other details appear about the same as after the usual or aqueous periodic acid-Schiff's procedure.)

Sections stained for dextran should be compared with control duplicate sections exposed to water either during or prior to periodic acid oxidation. We stain control sections by the aqueous periodic acid-Schiff's routine. The method used follows: Pass sections through xylenes and alcohols to water; treat sections for 10 minutes in either 1% periodic acid in water or in 0.8% potassium metaperiodate (KIO_4) in 0.3% HNO_3; wash in tap water and complete as directed in the method for dextran, starting with step 4. Dextran is dissolved by aqueous periodic acid and, therefore, will be absent in control sections (compare Figs. 5 and 6). Sites of very heavy deposits of dextran may be faintly pink after aqueous periodic acid-Schiff's staining. This faint coloration is prevented if sections containing large amounts of dextran are left standing in water for some time prior to staining.

PART II: EXPERIMENTAL STUDIES

In our earlier report,[16] we described the finding of abundant dextran in renal tubules and in liver cells of both normal mice and shocked mice killed 5 hours after a single 1 ml. injection of 6 per cent dextran given intravenously. Using the much improved method for the histochemical study of dextran, we have extended these observations with regard to normal mice to include the study of various tissues, the influence of the time after injection, and the effects of changing the dose of dextran.

Materials and Methods

White female mice (18 to 22 gm.) of the N.I.H. colony were used for all studies. Both dextran* and macrodex† were used as 6 per cent solutions in 0.9 per cent NaCl. Injections were made slowly into the

* Obtained from Commercial Solvents Corp., Terre Haute, Ind.
† Obtained from Pharmacia, Ltd., Uppsala, Sweden.

lateral tail vein. No reactions to either dextran preparation were seen. Mice were killed and necropsied at stated intervals after injection. The first group of mice were decapitated. Later, mice were killed with ether in order to avoid loss of blood from the tissues. Tissues were fixed, cut, and stained as prescribed for the study of dextran.

Experiments

A. Twenty-one normal mice were each injected with 1 ml. of 6 per cent dextran (Commercial Solvents). Mice were killed at varying intervals after injection in order to assess the influence of time alone on the amount of histochemically demonstrable dextran. In addition to kidney and liver, the spleen, lung, and pancreas were collected. Mice were killed at the following intervals after injection: one mouse each hour from the second to the seventh hour; 5 mice at 12 hours; 4 mice at 24 hours; 3 mice at 48 hours; and 3 mice at 96 hours. Food but not water was withheld from 16 hours prior to injection until 36 hours afterward.

B. The first experiment showed that various tissues contained dextran and did so for as long as 4 days after injection. Next, we wanted to know if larger doses would result in even greater quantities of histochemically demonstrable dextran that might persist as long as 1 month or more after injection. Therefore, 13 normal mice were each given a total of 9 ml. of dextran (Swedish dextran or macrodex), given as successive daily doses of 1 ml. except that 2 days elapsed between the fourth and fifth injections. After being fasted for 18 to 24 hours, 2 mice were killed for study at each of the following intervals after injection: 5 hours, 2 days, 4 days, 7 days, 12 days, and 30 days. The last mouse was killed 3 months after completion of dextran injections, following a 24-hour fasting period. Each of the tissues previously mentioned, together with myocardium, mesenteric lymph node, and fat, were taken for study.

C. As experiment *B* showed that dextran may persist in tissues for several months when large doses were used, we now wished to study the *storage* of dextran at reduced levels of dosage. Six mice were each given 1 ml. of dextran (Commercial Solvents), an amount comparable to what might be used clinically for the treatment of severe shock. A second group of mice were each given two 1 ml. injections, 1 hour apart. A third group of mice were each given three 1 ml. injections of dextran, 1 hour apart. A dose of 3 ml. in a 20 gm. mouse is comparable to the dosage of dextran used by Bull and his co-workers[3] in their serologic and chemical study of dextran storage in the rabbit. Two

mice from each dose-group were killed at 1, 2, and 3 months after injection. In every case mice were fasted 24 hours prior to sacrifice. Tissues examined were the same as in experiment *B* except for the addition of skin.

D. To determine the possible natural occurrence of any materials having staining properties similar to dextran, 20 mice not given dextran were killed for histochemical study. Half of the group were fasted 24 hours prior to death. Tissues taken for study were the following: Liver, kidney, spleen, lung, myocardium, pancreas, mesenteric lymph node, and fat. From 5 additional mice, blocks of adrenal gland and skin were examined. Tissues were processed and stained for dextran by the same technique as employed for the dextran-treated animals.

Results

Experiment A: Influence of Time, Up to 96 Hours, on the Distribution of Dextran-Like Material in Various Tissues after a Single (1 ml.) Intravenous Injection of Dextran

Tissue sections stained for dextran were compared with control sections stained by the aqueous periodic acid-Schiff's routine. In this manner, the amount of dextran-like material in each specimen of a particular organ was graded. Thus, the assigned gradings show the amount and distribution of dextran-like material in that organ with increasing time after injection. The gradings only approximately reflect the respective amounts of dextran-like material in the various organs of an individual mouse. Table I summarizes the distribution by organs and amounts of dextran-like material relative to time after dextran injection.

The lumina of *blood vessels* in all organs contained varying amounts of fine to coarse, extracellular granules of dextran-like material. The amount was greatest soon after injection and disappeared by 48 hours. The histologic estimation of dextran-like material in blood is limited by blood loss from tissues at necropsy.

The *liver* in all mice contained dextran in parenchymal cells (Fig. 1). As soon as 2 hours after injection, the cytoplasm of liver cells possessed many rounded and homogeneous masses that were deep purple after dextran staining but absent in control sections. There were only cytoplasmic vacuoles to account for the dextran-like material in sections stained by the aqueous method. Dextran-like material was still present in liver cells after 24 hours but was less in amount. There was little glycogen in liver cells until mice were given food

again. After feeding, there was so much glycogen in liver cells that dextran-like material was obscured and difficult to grade.

Kupffer cells of the liver contained granules of dextran-like material as early as 4 hours after injection. The amount of phagocytosed material increased and seemed maximal by 24 hours. Some variation in Kupffer cell content of phagocytosed dextran-like material was noted

TABLE I

Distribution and Amount of Dextran-like Material in Various Tissues of the Mouse after a Single (1 ml.) Intravenous Injection of Dextran

		All organs	Liver			Kidney			Spleen	Lung	Pancreas
Hours after dose	Specimen no.	Lumina of blood vessels	Liver cells	Kupffer cells	(Glycogen content)	Tubular epithelium	Tubular lumina	Phagocytes	Phagocytes of red pulp	Phagocytes	Phagocytes
2	1	3	2	0	1	1	1	0	0	0	0
3	2	3	3	0	1	2	1	0	0	0	0
4	3	3	3	1	1	1	1	0	0	0	0
5	4	3	3	1	Trace	1	1	1	0	Trace	1
6	5	3	3	1	2	1	1	1	0	Trace	0
7	6	3	3	1	0	1	1	2	1	Trace	2
12	7	2	3	2	1				?	Trace	2
	8	2	3	2	0	0	0	2	?	Trace	1
	9	2	3	3	Trace	0	Trace	2	1	1	0
	10	2	3	2	0	0	Trace	Trace	2	2	1
	11	2	2	3	0				2		2
24	12	1–2	2	3	Trace	0	0	Trace	2	3	2
	13	1	3	1	1	Trace	Trace	2	1	2	2
	14	2	2	3	Trace	0	0	2		2	2
	15	1–2	1	3	0	Trace	Trace	2	2	1	3
48	16	0	*	2	3	0	0	2	2	2	3
	17	0	*	3	4	0	0	2	2	1	2
	18	0	*	3	4	0	0	2	2	2	3
96	19	0	*	2	2	0	0	2	1	3	3
	20	0	*	2	3	0	0	2	1	1	1
	21	0	*	2	2	0	0	1	1	1	1

Schema for grading amounts of dextran-like material and also glycogen: 4, abundant; 3, moderate; 2, slight; 1 minimal; and trace, usually focal. Equivocal amounts of dextran-like material are indicated by a question mark.

* Dextran-like material present but greatly obscured by glycogen. (Animals 16 to 21 not fasted before death.)

in individual mice. Considerable dextran-like material was still present in Kupffer cells after 96 hours.

Kidneys from most mice killed in the first 24 hours contained small amounts of dextran-like material in the lumina and lining cells of renal tubules. Casts and heavy cytoplasmic deposits of dextran-like material such as are seen in renal tubules of shocked mice were never encountered.[16] After the first few hours, phagocytes filled with granules of dextran-like material were always present. The phagocytes occurred

in the intertubular stroma and in the adventitia of large blood vessels. Even when renal tubules were empty, there was sometimes a coating of dextran-like material over the mucosa of the renal pelvis.

The *spleen* presented certain problems in regard to the detection of phagocytosed dextran-like material. As long as the content of such material in the blood was great, intracellular deposits were hard to discern. Also, red-brown pigment in reticular cells of the red pulp tended to obscure granules of dextran-like material. These troubles were overcome when larger doses of dextran were used, as in experiment B. Tissue sections of spleen from all mice killed more than 12 hours after injection contained definite deposits of dextran-like material in reticular cells of the red pulp.

In the *lung* it was difficult to detect dextran-like material in tissue cells during the first 12 hours because of the high concentrations of such material in the blood of the interalveolar septa. By 24 hours after injection, all lungs contained varying numbers of phagocytes laden with dextran-like material. These phagocytes were found around bronchi, arteries and veins, in septa, and in occasional alveoli. In staining control sections, the phagocytes were noted only as mononuclear cells with fairly abundant, finely vacuolated or "foamy" cytoplasm.

The *pancreas* contained distinctive, easily detected deposits of dextran-like material in stromal phagocytes as soon as 4 hours after injection. With few exceptions, the amount of phagocytosed material increased steadily for the first 48 hours. In control sections, the phagocytes were very inconspicuous. Toward the end of the experiment, the amount of phagocytosed dextran-like material decreased.

In summary, the liver seemed to contain the most dextran-like material. Both liver cells and Kupffer cells participated in the removal of dextran-like material from the blood. As long as 24 hours after dextran injection, little glycogen was found in liver cells of fasting mice. Neither was there any great decrease in content of dextran-like material in liver cells during this time. When the remaining mice were fed again, abundant amounts of glycogen were found in liver cells.

In kidney some dextran-like material was found in renal tubules, presumably undergoing excretion. Massive amounts of dextran-like material similar to those seen in shocked mice were not found. Study of the spleen was complicated by certain factors but did show some storage of dextran-like material in cells of the red pulp. Phagocytosed dextran-like material was seen also in lung and pancreas. Finally, while most tissues from mice killed 3 days after injection contained less dextran-like material than those killed earlier, the decrease was not great.

Experiment B: Distribution of Dextran-Like Material in Various Tissues of the Mouse after Multiple Intravenous Injections of Dextran (Total, 9 ml.)

The results of experiment B have been summarized in Table II. Definite and usually striking amounts of dextran-like material were demonstrable in every tissue specimen examined. For each organ the amounts of dextran-like material were graded by simultaneous com-

TABLE II

Distribution of Dextran-like Material in Various Tissues of the Mouse after Multiple (9 ml.) Intravenous Injections of Dextran

Organ and site of dextran-like material	Time interval after final injection of dextran						
	5 hours	2 days	4 days	7 days	12 days	1 month	3 months
Animal number	22 23	24 25	26 27	28 29	30 31	32 33	34
Blood vessels							
Contents of lumina	3 3	3 3	2 2	1 1	Trace 0	0 0	0
Liver							
Liver cells	5 5	* 5	4 2	4 3	2 1	0 1	0
Kupffer cells	5 5	5 5	5 5	5 5	5 5	4 4	3
Kidney							
Glomerular tufts	2 2	3 3	2 2	2 2	1 1	Trace Trace	0
Tubular epithelium	3 3	3 3	3 2	2 2	1 1	Trace Trace	0
Interstitial phagocytes	2 2	2 2	3 3	3 3	3 3	2 2	1
Spleen							
Phagocytes of red pulp	4 4	5 5	5 5	5 5	5 5	3 4	3
Lung							
Phagocytes	4 5	5 5	5 5	5 5	5 5	3 2	1
Myocardium							
Interstitial phagocytes	4 5	4 4	5	5 5	5 4	4 4	2
Pancreas							
Interstitial phagocytes	4 4	4	4	4	4 4	4 4	2
Lymph nodes							
Phagocytes of sinuses and medullary cords	4 4	4 5	5 5	4 5	5 4	5 5	4
Adipose tissue							
Interstitial phagocytes	3 4	3 3	4 3	3 3	3 3	5 4	2
Dextran-like material also observed in:							
Duodenum, phagocytes of lamina propria			2 2	2 2			
Phagocytes of ovary						3	
Phagocytes of endometrium						2 3	
Zona glomerulosa of adrenal gland						2	
Phagocytes of dermis and subcutis						4	2

Schema for grading amounts of dextran-like material: 5, marked; 4, abundant; 3, moderate; 2, slight; 1, minimal; and trace, usually focal.

* Abundant glycogen obscures dextran-like material.

parison of tissue sections from every animal. Grading was done on the basis of number and size of individual deposits, as follows: 5, marked; 4, abundant; 3, moderate; 2, slight; and 1, minimal.

In the *liver* extensive accumulations of dextran-like material were found in the cytoplasm of both liver cells and Kupffer cells just as observed on a smaller scale in the single-dose experiment A. During the first few days after completion of dextran injections, the cytoplasm of many liver cells was loaded with fine to coarse granules that were colored purplish black in sections stained for dextran (Fig. 3). These cytoplasmic granules were absent in control sections. After the first few days, the content of dextran-like material in liver cells gradually declined (Figs. 4 and 5). One month after the last dextran injection, there was little or no dextran-like material in liver cells. On the other hand, deposits of dextran-like material in Kupffer cells appeared maximal throughout the first 12 days (Fig. 5) and only slightly decreased even after 1 month. Mouse 34 was killed 3 months after the last injection of dextran and still showed moderate amounts of dextran-like material in Kupffer cells (Fig. 15).

The *kidneys* of all animals contained only minimal to moderate amounts of dextran-like material. Coarse and fine granules were present in glomerular tufts up to 1 month after injections. In most cases it was our impression that the granules of dextran-like material were within capillary loops and not definitely inside glomerular epithelial cells. The coloration of the dextran-like material was so intense that the adjacent endothelial and epithelial cells were not well seen. Both coarse and fine granules of dextran-like material were observed also in cells lining the proximal convolutions and, to a lesser extent, other portions of the renal tubules (Fig. 2). The lumina of renal tubules frequently contained granules and globules of dextran-like material. However, renal tubules never showed widespread formation of casts of the type observed in shocked mice given only a single injection of dextran. Numerous phagocytes filled with dextran-like material usually were present in the intertubular stroma and in the adventitia of arteries and veins. These phagocytes, present in minimal number for as long as 3 months, were not limited to any particular level of the nephron.

The *spleen* in all animals contained phagocytosed dextran-like material confined to the cells of the red pulp, sharply outlining the lymphoid follicles (Fig. 9). No definite decrease in phagocytosed dextran-like material was evident during the first 12 days. Some decrease was evident after 1 month, but moderate amounts of dextran-like material were present as long as 3 months after completion of dextran injections (Fig. 10).

Lungs of all animals contained widely distributed phagocytes laden with dextran-like material. These phagocytes were present in the septa, around bronchi and larger blood vessels, and inside both alveoli and bronchi (Fig. 7). In control sections the phagocytes appeared as mononuclear cells with abundant, finely vacuolated or foamy cytoplasm. By 3 months after the injections of dextran, these phagocytes had undergone a considerable decrease in number.

The *myocardium* of the left ventricle, not previously studied, was found to contain numerous, distinctively distributed phagocytes filled with dextran-like material in all animals killed during the first month. The phagocytes, situated between muscle fibers, were very conspicuous in sections stained for dextran, but hardly discernible after other staining methods. Phagocytes containing dextran-like material appeared reduced in both number and size in the last animal killed, 3 months after the injections. Dextran-like material was never observed within muscle fibers.

Every *pancreas* examined contained deposits of dextran-like material similar in distribution to those found in experiment A (Fig. 8). The individual masses of dextran-like material were, however, larger and denser than those seen after only 1 ml. of dextran. The amount of demonstrable dextran-like material seemed more or less constant during the first month but was definitely reduced by the third month after the last injection (Fig. 14).

Lymph nodes of all mice contained dextran-like material, both in phagocytes and often in the free lymph. Often the subcapsular sinuses contained fairly numerous free and lining cells filled with dense, purple-black, coarse and fine granules in sections stained for dextran (Fig. 11). In addition, there were usually some similar phagocytes within medullary cords but never within lymphoid follicles. Unlike most of the tissues studied, no decrease in content of dextran-like material was evident during the period of observations. Abundant dextran-like material was present even after 3 months (Fig. 13).

Adipose tissue contained somewhat variable amounts of phagocytosed dextran-like material. Large amounts were found as long as 1 month after the last injection (Fig. 12). Dextran-like material was not present in fat cells but in small mononuclear cells situated between the fat cells.

Dextran-like material was observed incidentally in a small number of other tissues. In several specimens the lamina propria of the duodenum was seen to contain fairly numerous phagocytes laden with dextran-like material. The stroma of the ovary in one animal contained moderately numerous dextran-laden phagocytes. Similar phagocytes were observed in the stroma of the endometrium in two instances.

Both adrenal glands of one animal were found to contain fine granules of dextran-like material in the cytoplasm of nearly all cells of the zona glomerulosa. In contrast, the other layers of the adrenal cortex did not contain any dextran-like material. In the medullary portions, however, there were moderate numbers of phagocytes containing dextran-like material. Finally, in sections of skin from 2 animals (33 and 34) there were phagocytes filled with dextran-like material scattered throughout the dermis and subcutis (Fig. 16). No dextran-like material was observed in skeletal muscle accompanying the portions of skin.

Experiment C: Comparison of the Storage of Dextran-Like Material after Graded Doses of Dextran, Up to Three Months after Injection

The findings in experiment C have been summarized in Table III. The distribution of dextran-like material was similar to that previously described. Phagocytes loaded with coarse granules of dextran-like material were found in the stroma of the kidney, lung, myocardium, pancreas, adipose tissue, and in the dermis and subcutis of skin. Dextran-like material also was present in the Kupffer cells, reticular cells of the red pulp in the spleen, and in both the free phagocytes and the lining cells of lymph node sinuses.

Regardless of the time after injection, the total amount of dextran-like material demonstrated was roughly proportional to the amount of dextran injected. In respect to both Kupffer cells and phagocytes of the myocardium and pancreas, the number of phagocytic cells appeared to be increased less than did the amount of dextran-like material in individual phagocytes. In the other organs both the number of phagocytes and the amount of phagocytosed material seemed greater after larger doses of dextran.

Whether the amount of dextran injected was 1 or 3 ml., the amount of dextran-like material in tissues steadily but slowly declined with increasing time after injection. The amount of dextran-like material in individual Kupffer cells and phagocytes of myocardium and pancreas appeared to decline with increasing post-injection interval. In other tissues examined at successively longer intervals after injection, the number of phagocytes containing dextran-like material appeared to decrease more than the amount contained in each cell.*

* Five mice were examined 8 months after the injection of 2 to 3 ml. of dextran. No dextran-like material was found in liver, spleen, or lymph nodes. However, small numbers of phagocytes laden with dextran-like material were still present in the pancreas and mesenteric fat of each mouse.

TABLE III

Dextran-like Material in Tissues of the Mouse after Graded (1-3 ml.) Doses of Intravenous Dextran

		One month after injection of dextran					
		1 ml.		2 ml.		3 ml.	
	Animal no.	35	36	37	38	39	40
Site of dextran-like material	Phagocytes of						
	Kidney	0	0	0	0	2	1
	Spleen	0	1	2	3	4	4
	Lymph nodes	4	4	4	4	4	4
	Lung	Trace	0	Trace	1	Trace	2
	Myocardium	0	2	3	3	4	3
	Pancreas	2	2	4	3	4	4
	Fat	2	2	3		4	3
	Skin	1	3	4	3	4	4
	Liver cells	0	0	0	0	0	0
	Kupffer cells	2	2	3	3	4	4
		Two months after injection of dextran					
		1 ml.		2 ml.		3 ml.	
	Animal no.	41	42	43	44	45	46
Site of dextran-like material	Phagocytes of						
	Kidney	0	0	0	Trace	0	0
	Spleen	0	0	2	2	3	4
	Lymph nodes	4	0	3	4	4	3
	Lung	2	1	1	1	0	3
	Myocardium	Trace	Trace	2	2		2
	Pancreas	1	2	3	2	3	3
	Fat	1	1	3	3	3	3
	Skin	3	3	3	3	4	4
	Liver cells	0	0	0	0	0	0
	Kupffer cells	0	1	3	3	3	4
		Three months after injection of dextran					
		1 ml.		2 ml.		3 ml.	
	Animal no.	47	48	49	50	51	52
Site of dextran-like material	Phagocytes of						
	Kidney	0	0	0		0	0
	Spleen	0	0	Trace?	1	2	2
	Lymph nodes	2	0	3	3	3	3
	Lung	0	0	0		0	0
	Myocardium	0	0	Trace	Trace	2	2
	Pancreas		Trace	2	1	2	2
	Fat		Trace	3	2	2	3
	Skin	2	2	2	3	2	3
	Liver cells	0	0	0	0	0	0
	Kupffer cells	0	0	0	0	2	1

Schema for grading deposits of dextran-like material: 4, abundant; 3, moderate; 2, slight; 1, minimal; and trace, usually focal.

The content of dextran-like material in Kupffer cells, among the various sites studied, most consistently reflected changes in either the amount of dextran injected or in the time elapsing after injection. Lymph node sinuses contained an amount of dextran-like material

that seemed maximal after only 1 ml. of dextran. Thus the influence of larger doses of dextran was not well shown by the study of lymph nodes.

In spite of trebling the dose of dextran, no dextran-like material was present in liver cells at 1 month after injection.

Experiment D: Control Studies of Tissues from Normal Mice Not Given Dextran

Dextran-like material was not present in the following tissues from 20 mice of experiment D: liver, kidney, spleen, lung, myocardium, pancreas, mesenteric fat, and lymph nodes. Skin and adrenal glands contained no dextran-like material in 5 mice in which these tissues were examined. Naturally occurring material with the staining properties of dextran was encountered in the stomach. The gland-neck mucin of the mouse stomach is deeply colored by Schiff's reagent after periodic acid in alcohol but not after periodic acid in water. So this particular mucin resembles dextran in being intensely periodic acid-Schiff positive but soluble in water unless previously oxidized by periodic acid in alcohol. While this example causes no difficulty, it illustrates that naturally occurring dextran-like materials exist and may be mistaken for dextran unless tissues of control animals are studied.

Glycogen was either minimal or absent in livers of all fasted mice. Glycogen present in livers of non-fasted mice appeared just as abundant after aqueous periodic acid-Schiff's staining as after the alcoholic periodic acid-Schiff's routine for dextran. In fixed tissue, liver glycogen is insoluble in water.

Discussion

Dextran-like material was demonstrated in tissue sections of one or more of a series of tissues from each of 52 dextran-treated mice killed at varying intervals after injection. When the same tissues were taken from each of 20 mice not given dextran and examined by the same technique, no dextran-like material was found. On the basis of this evidence, we believe the material demonstrated in tissues of dextran-treated mice to be dextran. From histochemical study it is impossible to say that no degradation of dextran occurs in tissues. Therefore, we regard the material demonstrated as *incompletely hydrolyzed dextran* only. The ultimate product of dextran hydrolysis should be glucose. The methods used do not demonstrate glucose in tissue sections.

Intravenously injected dextran was demonstrated in blood, renal tubules, liver cells, and in widely scattered phagocytes composing the

reticulo-endothelial system. Dextran was present extracellularly in the lumina of all blood vessels in gradually decreasing amounts during the first 48 hours after injection. The histochemical estimation of dextran in blood is limited by variations in the loss of blood when tissue blocks are taken at necropsy.

Dextran is present in renal tubules very soon after injection. It is found both in the lumina and in the cytoplasm of cells lining the proximal convoluted tubules and, to a lesser extent, in other portions of the tubules. Compared to the amount of dextran known to be excreted in the urine of normal mice, namely, about 30 per cent or so, the amount of histochemically demonstrable dextran in renal tubules seems small indeed.[6] Previous studies[16] showed much greater amounts of dextran in both the lumina and in lining cells of renal tubules in shocked mice compared to normal mice given the same dose. Yet, quantitative chemical studies show much greater urinary excretion of dextran in normal mice than in shocked mice.[6] This suggests that the amount of dextran demonstrated in renal tubules is more closely related to the concentration of dextran in the urine than to the total amount of dextran being eliminated.

Dextran can be demonstrated in the cytoplasm of liver cells as soon as 2 hours after injection and appears to be about maximal in 12 to 24 hours. Even when mice are fasting, deposits of dextran in liver cells appear almost unchanged after 48 hours. However, even after quite large doses, no dextran is seen in liver cells at the end of 1 month. Dextran in liver cells tends to disappear and may be metabolized.

Dextran is removed from the blood not only by the parenchymal cells of the liver but also by the Kupffer cells. Beginning phagocytosis is evident as soon as 4 hours after injection. The amount of dextran in Kupffer cells increases and appears maximal by 24 hours after injection. Dextran in Kupffer cells persists much longer than in liver cells. Some dextran was still present in the Kupffer cells in one of 2 mice killed 2 months after each had received 1 ml. of dextran, as a single dose. After larger doses of dextran, a correspondingly greater amount of dextran is demonstrable in Kupffer cells and for a longer time after injection. Dextran was still present in several mice killed 3 months after injection. When the size of the liver is considered, the amount of dextran taken up by liver cells and Kupffer cells may represent a significant proportion of the injected dose.

Deposits of dextran are found also in phagocytes of lymph nodes, spleen, pancreas, and myocardium. Smaller, but sometimes large, numbers of phagocytes were seen in fat, lung, kidney, and skin.

Phagocytosis of dextran was evident in various sites as soon as 6 to 12 hours after injection. Months later, dextran was present in the same sites but reduced in amount. The histochemical findings confirm those obtained with the serologic method by Bull *et al.*[3]

The persistence of dextran within phagocytes argues against the view that these cells degrade or metabolize dextran to any extent. The gradual decrease in phagocytosed dextran that has been observed may come about in several ways. Dextran-laden phagocytes may be slowly lost by migration across various mucosal surfaces into secretions and excretions of the body. Or, phagocytes may die and lose part of their dextran to the urine. Finally, the appearance of a decrease in phagocytosed dextran might result from the dispersion of dextran-loaded phagocytes throughout the various tissues of the body.

The total amount of dextran present in reticulo-endothelial cells months after injection is probably quite small. Numerous dextran-laden phagocytes have been found in various tissues shortly after injections of only 2 to 4 mg. of dextran in mice.[23] This means that the histochemical method for dextran is exceedingly sensitive. Even though small in amount, dextran stored in reticulo-endothelial cells may be significant from an immunologic standpoint.

Whether the storage of dextran by reticulo-endothelial cells is deleterious can be determined only by much further study.

We believe dextran may be useful for studies of the reticulo-endothelial system. Dextran itself appears virtually innocuous and can be used in almost any amount. There is hope that dextrans of varying molecular weight will be available presently. Dextran can be intensely colored by a simple method. In duplicate sections, histologic features can be studied without interference by any natural color of the stored material. Finally, such studies would add not only to our knowledge of the reticulo-endothelial system but also to the specific question about the safety of dextran for clinical use.

Summary

Dextran was stained in tissue sections by an alcoholic periodic acid-aqueous Schiff's method. Duplicate sections stained by the aqueous periodic acid-Schiff's method failed to show dextran. Naturally occurring dextran-like materials were excluded by the study of tissues from mice not given dextran.

Dextran was demonstrated in liver cells, renal tubules, and in widely scattered phagocytes composing the reticulo-endothelial system. Even when large doses of dextran were used, there was little or no dextran

left in liver cells after 1 month. Dextran was found in the lumina of renal tubules only for the first day or so, during the period of active urinary excretion. Dextran granules in the cytoplasm of renal epithelium persisted longer, but were largely gone by 2 to 4 weeks after injection. However, dextran phagocytosed by reticulo-endothelial cells decreased more slowly and was still present months after injection.

REFERENCES

1. Ingelman, B. Investigations on dextran and its application as a plasma substitute. *Upsala läkaref. förh.*, 1949, **54**, 107–122.
2. Thorsén, G. Dextran as a plasma substitute. *Lancet*, 1949, **1**, 132–134.
3. Bull, J. P., Ricketts, C., Squire, J. R., Maycock, W. d'A., Spooner, S. J. L., Mollison, P. L., and Paterson, J. C. S. Dextran as a plasma substitute. *Lancet*, 1949, **1**, 134–143.
4. Hehre, E. J., and Neill, J. M. Serological differences among native and clinical dextrans. *Federation Proc.*, 1952, **11**, 471.
5. Bloom, W. L., and Willcox, M. L. Determination of dextran in blood and urine. *Proc. Soc. Exper. Biol. & Med.*, 1951, **76**, 3–4.
6. Millican, R. C., and Mowry, R. W. Histochemical and urinary excretion studies of dextrans in normal and shocked mice. *Federation Proc.*, 1953, **12**, 352.
7. Goldenberg, M., Crane, R. D., and Popper, H. Effect of intravenous administration of dextran, a macromolecular carbohydrate, in animals. *Am. J. Clin. Path.*, 1947, **17**, 939–948.
8. Turner, F. P., Butler, B. C., Smith, M. E., and Scudder, J. Dextran, an experimental plasma substitute. *Surg., Gynec. & Obst.*, 1949, **88**, 661–675.
9. Nelson, A. A., and Lusky, L. M. Pathological changes in rabbits from repeated intravenous injections of periston (polyvinyl pyrrolidone) or dextran. *Proc. Soc. Exper. Biol. & Med.*, 1951, **76**, 765–767.
10. Gray, I., Siiteri, P. K., and Pulaski, E. J. Metabolism of plasma substitute I. Dextran (macrodex). *Proc. Soc. Exper. Biol. & Med.*, 1951, **77**, 626–627.
11. Cargill, W. H., and Bruner, H. D. Metabolism of C^{14} labeled dextran in the mouse. *J. Pharmacol. & Exper. Therap.*, 1951, **103**, 339.
12. Terry, R., and Yuile, C. L. Fate of intravenous C^{14} labeled dextran in dogs. *Federation Proc.*, 1952, **11**, 430.
13. Engstrand, L., and Åberg, B. Excretion of intravenously administered dextran. *Lancet*, 1950, **1**, 1071–1073.
14. Schmitz, H. Ueber das Schicksal des Macrodex im Organismus. *Klin. Wchnschr.*, 1951, **29**, 424–425.
15. Hartman, F. W. Tissue changes following the use of plasma substitutes. *Arch. Surg.*, 1951, **63**, 728–738.
16. Mowry, R. W., Longley, J. B., and Millican, R. C. Histochemical demonstration of intravenously injected dextran in kidney and liver of the mouse. *J. Lab. & Clin. Med.*, 1952, **39**, 211–217.
17. Hotchkiss, R. D. A microchemical reaction resulting in the staining of polysaccharide structures in fixed tissue preparations. *Arch. Biochem.*, 1948, **16**, 131–141.
18. McManus, J. F. A. Histological demonstration of mucin after periodic acid. *Nature, London*, 1946, **158**, 202.
19. Lillie, R. D. Studies on the preservation and histologic demonstration of glycogen. *J. Tech. Methods*, 1947, **27**, 23–61.

20. Friberg, U., Graf, W., and Åberg, B. On the histochemistry of partly hydrolysed, bacterial dextran. *Scandinav. J. Clin. & Lab. Invest.*, 1951, **3**, 221–227.
21. Mowry, R. W. Histochemical methods for water-soluble, alcohol-insoluble polysaccharides and other substances oxidized to aldehydes by periodic acid. *J. Nat. Cancer Inst.*, 1952, **13**, 230–231.
22. Lillie, R. D. Simplification of the manufacture of Schiff reagent for use in histochemical procedures. *Stain Technol.*, 1951, **26**, 163–165.
23. Mowry, R. W., and Millican, R. C. Unpublished data.

LEGENDS FOR FIGURES

In the photographs, deposits of dextran appear black. Save for Figure 6, all sections were colored by the alcoholic periodic acid-aqueous Schiff's method. Tissues shown in Figures 2 to 16 are from experiment B in which mice were each given nine 1 ml. injections or a total of 9 ml. of clinical dextran.

FIG. 1. Liver of a mouse 5 hours after a single 1 ml. injection of dextran. Dextran is seen in liver cells and blood vessels. No glycogen is present. × 230.

FIG. 2. Kidney, 5 hours after the last injection of dextran. Some dextran is seen in the cytoplasm of cells lining certain proximal convoluted tubules. × 230.

FIG. 3. Liver, 5 hours after the last injection. Granules of dextran fill the cytoplasm of many liver cells and Kupffer cells. No glycogen is present. × 96.

FIG. 4. Liver, 4 days after the last injection. Somewhat less dextran is seen in liver cells, as compared to Figure 3. Kupffer cells are still full of dextran. No glycogen is present. × 96.

FIG. 5. Liver, 12 days after the last dose of dextran. Most of the dextran shown is within Kupffer cells. Little dextran remains in liver cells. No glycogen is present. × 96.

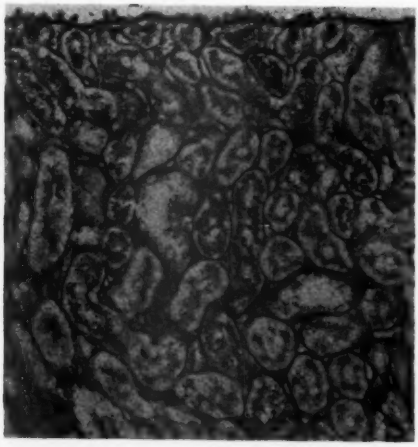
FIG. 6. Liver, duplicate section from the same block as the section used in Figure 5, but stained by the aqueous periodic acid-Schiff's method. Dextran is dissolved by the aqueous reagent and therefore is absent. This type of control is always used. × 96.

1

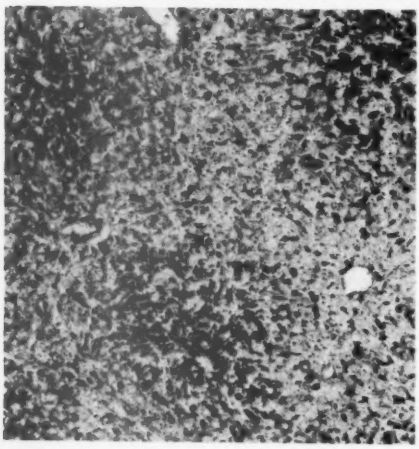
3

5

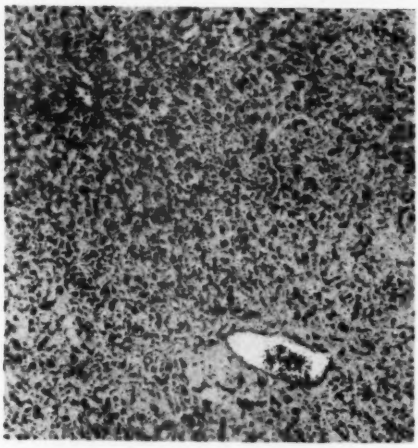
1



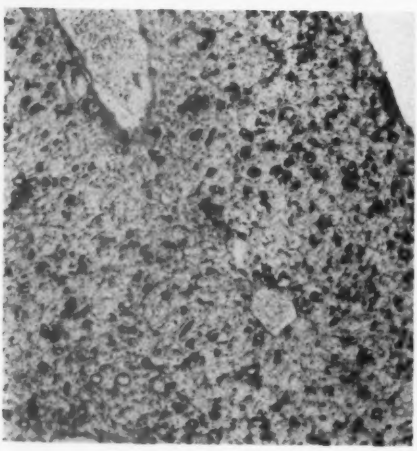
2



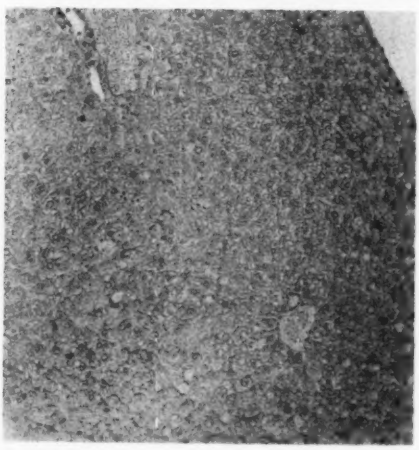
3



4



5



6

FIG. 7. Lung, 12 days after the last dose of dextran. Phagocytes loaded with dextran appear black in the photograph. × 96.

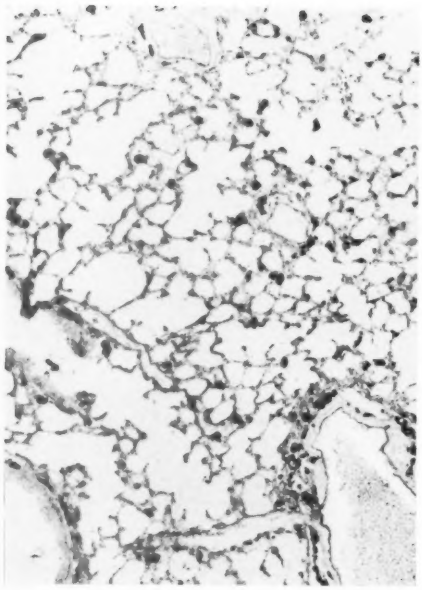
FIG. 8. Pancreas, 12 days after the last dose of dextran. Phagocytes loaded with granules of dextran are present in the stroma and, to the right, in the adventitia of an artery. × 230.

FIG. 9. Spleen, 4 days after the last injection. Dextran in reticular cells of red pulp appears black and outlines the follicles. × 46.

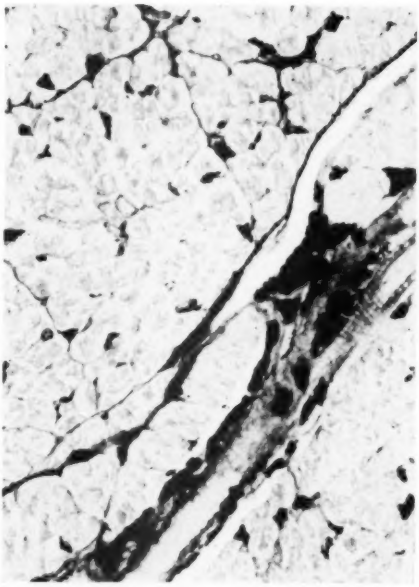
FIG. 10. Spleen, 3 months after the last dose of dextran. The amount of phagocytosed dextran appears reduced as compared to Figure 9. × 46.

7

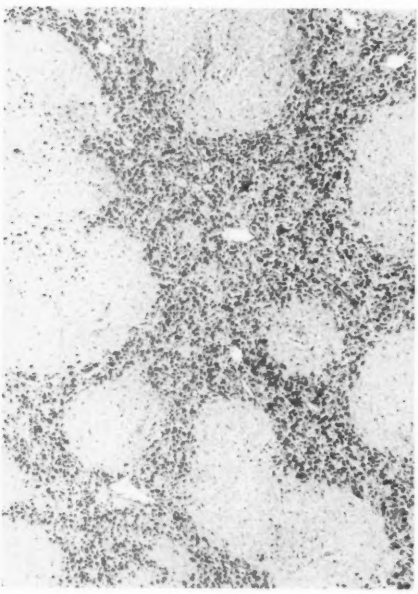
5



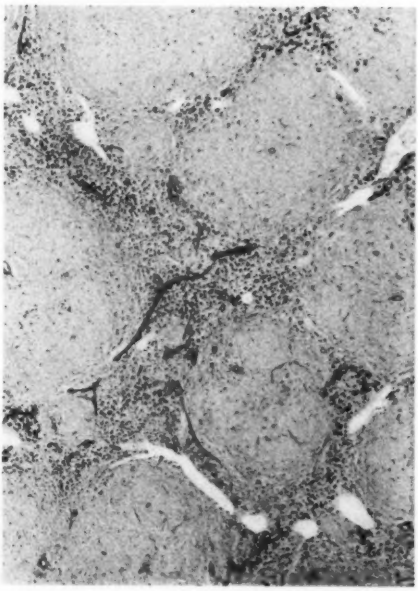
7



8



9



10

FIG. 11. Mesenteric lymph node, 4 days after the last dose of dextran. Numerous phagocytes filled with dextran are seen in the subcapsular sinuses but not in the follicles. Dextran-laden phagocytes are also seen in the fatty tissue. × 46.

FIG. 12. Mesenteric fat, 1 month after the last dose of dextran. Phagocytes loaded with dextran are numerous. × 46.

FIG. 13. Lymph node, 3 months after the last injection of dextran. Phagocytes filled with dextran are seen in sinuses and medullary cords but not in follicles at lower left. × 230.

FIG. 14. Pancreas, 3 months after the last injection of dextran. Phagocytes loaded with dextran are still present. For comparison with Figure 8. × 230.

FIG. 15. Liver, 3 months after the last dose of dextran. For comparison with Figure 5. Dextran is reduced but still present in occasional Kupffer cells and in phagocytes about a vein shown in the lower portion of the field. Glycogen is present in many liver cells and appears gray to gray-black in the photomicrograph. × 96.

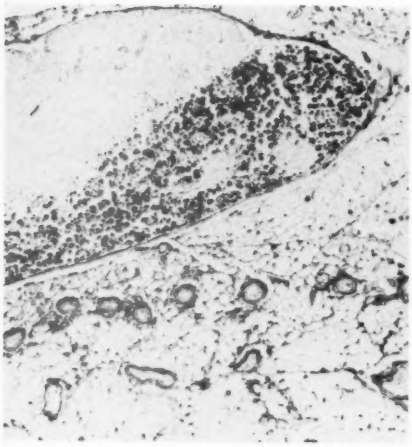
FIG. 16. Skin, 3 months after the last dextran injection. Small numbers of dextran-laden phagocytes are seen in the dermis and subcutis. × 185.

11

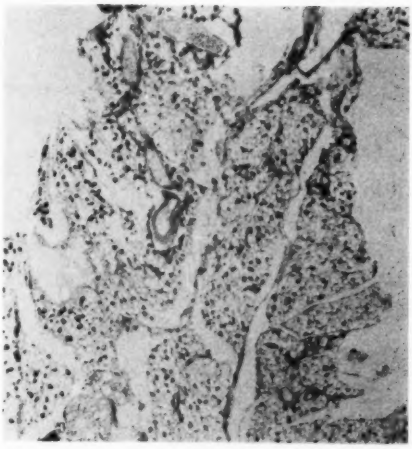
13

15

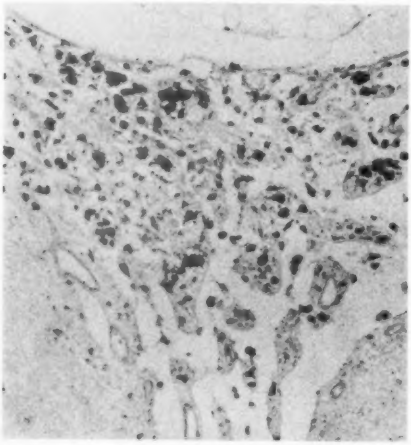
11



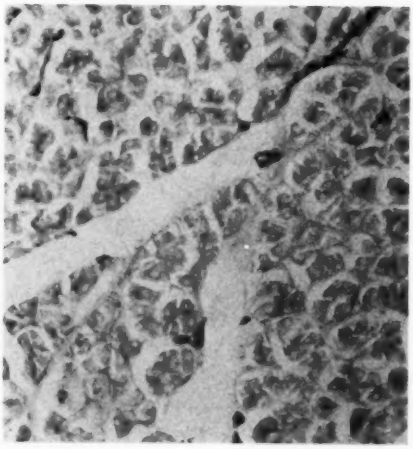
12



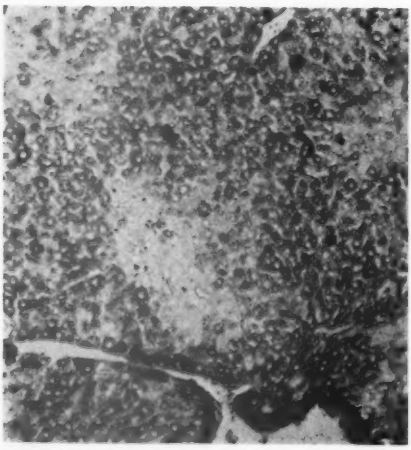
13



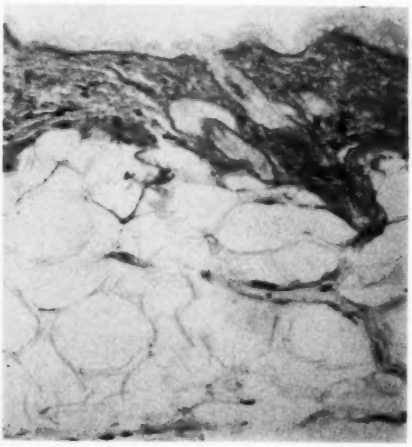
14



15



16



EXPERIMENTAL INVESTIGATION OF CHANGES IN AXIS CYLINDERS OF PERIPHERAL NERVES FOLLOWING LOCAL COLD INJURY*

W. J. Pirozynski, M.D., and D. R. Webster, M.D.

(From the Department of Experimental Surgery, McGill University, Montreal, Que.)

This study was undertaken to investigate the effects of cold on the axon structures in peripheral nerves, and to gain more knowledge concerning the pathogenesis of local cold injury, since it is well known that the application of cold is capable of producing irreversible damage in various living tissues. The sequence and nature of the degeneration appearing in the various fibers of peripheral nerves were studied also.

Material and Methods

Adult male albino rabbits, each weighing more than 2500 gm., were used. They were anesthetized by sodium pentobarbital injected intraperitoneally.

One ear was cooled to a temperature between −20° C. and −30° C. by immersing it in a fluid medium maintained at this temperature level. The fluid contained ethylene glycol, alcohol, and water, and was cooled by means of solid carbon dioxide. After solidification, which took place in 30 to 70 seconds, the ear was kept immersed in the cold bath for another 2 to 4 minutes. At the end of this period the ear was removed from the cold bath and allowed to thaw in room air at approximately +22° C. Thawing and re-establishment of the circulation were complete within 20 minutes. The opposite ear of the animal served as the control.

Since specific methylene blue staining of axons is capable of producing histologic pictures comparable with those obtained by selective silver methods,[1] the intravital staining technique (according to the procedure introduced by Weddell and Glees[1]) was used exclusively. Five to 7 cc. of 0.01 per cent solution of methylene blue chloride in 0.9 per cent sodium chloride were injected subcutaneously into the dorsum of the injured and control ears. The dye was injected at intervals after injury varying from 2 to 72 hours, allowing 30 to 45 minutes for staining. The animals were then sacrificed by intracardiac injections of ether; the stained skin was gently stripped from the aural cartilage and fixed in 8 per cent solution of ammonium molybdate at a temperature between 0° and +4° C. for a period of 8 to 12 hours. The specimens were then washed in ice-cold 0.9 per cent sodium chlo-

* This work was supported by a grant-in-aid from the Defense Research Board of Canada.

Received for publication, December 5, 1952.

ride solution for 30 to 60 minutes, dehydrated in 95 per cent and absolute alcohol, cleared in toluene, and mounted in permount.*

Control experiments were carried out on burned and ischemic ears in order to observe the effects of other physical agents and anoxia on peripheral nerve fibers. The burns were produced by immersing one ear in water at a temperature of +65° C. for 4 minutes. Ischemia was produced according to the method of Pochin[2] by occluding the circulation by means of a strip of rubber wound around the ear, after placing a rubber stopper in the ear. The cessation of circulation was confirmed by a drop in skin temperature and complete stasis in visible blood vessels without engorgement of the veins. The period of ischemia corresponded to the duration of exposure to cold plus the total time required for thawing, *i.e.*, between 20 to 30 minutes. Specimens of burned and ischemic skin were taken at intervals of 24 and 48 hours after injury.

Observations

Three groups of nerve fibers were distinguished; namely, coarse medullated fibers, fine medullated fibers, and non-medullated fibers. In normal skin both medullated and non-medullated fibers were observed (Figs. 1, 2, and 3). The medullated (Fig. 2) could be distinguished from the non-medullated fibers (Fig. 1) by their larger diameter and the presence of the nodes of Ranvier. The internodal portions in medullated and the axons in non-medullated fibers generally were smooth and homogeneous, although changes in diameter due to indentation of Schwann's sheath were present.[1] There was a progressive decrease in diameter of the nerve fibers from the nerve plexus to their terminations. Occasionally a faint outline of a myelin sheath could be noted (Fig. 2). The technique did not make visible the Schwann nuclei or the Schmidt-Lantermann clefts. In some specimens normal fibers showed interruptions of axons at internodal portions, but these were probably artefacts.[1] In normal skin a small number of axis cylinders in all nerve fibers, and in the cutaneous nerve plexuses, showed signs of degeneration, consisting of axonal disintegration, comparable with axons in wallerian degeneration[1] (Fig. 3). Regenerating fibers also were present.

Two hours after cold injury numerous fibers showed various degrees of axonal swelling and irregularities in staining (Figs. 4 and 5). In the coarse medullated fibers fibrillation and fusiform swelling of axons were present at irregular intervals, especially at the nodes of Ranvier. Deviations in staining and marked swelling of axis cylinders also were observed in fine medullated and non-medullated fibers. The medullary sheaths became sharply outlined (Fig. 5). Many Schwann nuclei were

* Obtained from the Fisher Scientific Co., Montreal, Que.

deeply stained. A considerable number of coarse fibers, especially in nerve plexuses, did not show changes in the axon structure. Many fine medullated and non-medullated fibers were intact.

More extensive changes could be observed in sections taken between 4 and 6 hours after exposure. More fibers were affected. More axis cylinders of coarse medullated fibers showed marked swelling and fibrillation, although in the nerve plexus some fibers still showed no signs of degeneration (Fig. 6). The number of unaffected fine medullated and non-medullated fibers declined sharply 6 hours after exposure.

Almost all visible fibers presented signs of degeneration 12 hours after injury, except for some coarse fibers in the nerve trunk (Fig. 7). Fusiform swelling at internodal portions and increase in diameter at the nodes of Ranvier, as well as marked fibrillation, were seen in all fibers. Many fine medullated and non-medullated fibers showed axonal and granular disintegration (Fig. 8). The medullary sheaths were well outlined and the Schwann nuclei deeply stained.

Twenty-four hours after exposure there was extensive degeneration of axis cylinders in fibers of all sizes, although occasionally a few coarse fibers in the nerve plexus showing no signs of degeneration were still visible. Coarse and fine, medullated and non-medullated fibers presented granular disintegration or fragmentation of their axons (Fig. 9), while some of the coarse fibers still showed only swelling and fibrillation.

Forty-eight hours after exposure all fine fibers presented some degree of axonal disintegration, while a few coarse fibers, swollen and fibrillated, retained continuity of their axonal structures. Intact axons in coarse fibers were seen only occasionally. No morphologically intact axons could be found in the nerve fibers of specimens taken 72 hours after exposure (Fig. 10).

No abnormalities were found in specimens of skin taken between 24 and 48 hours after 20 to 30 minutes of total ischemia (Fig. 11).

In burned skin the axonal changes were quite similar to those in skin injured by cold. Twenty-four hours after exposure to heat all fine nerve fibers showed various degrees of degeneration, consisting of swelling, fibrillation, and fragmentation of axis cylinders, while coarse fibers contained intact as well as degenerating axons. Forty-eight hours after thermal injury, fibers of all sizes showed signs of degeneration in the form of fragmentation and disintegration of the axons (Fig. 12).

Comment

The morphologic changes observed in peripheral nerves following exposure to severe cold were recognized as progressive degeneration

of axis cylinders in all fibers. The earliest alterations in axonal structure consisted of various degrees of swelling at internodal portions and at the nodes of Ranvier, irregular fibrillation, and deviations in staining which were observed almost immediately after injury. The succeeding changes were characterized by axonal fragmentation and granular disintegration resulting in complete destruction within 48 to 72 hours after exposure. The final outcome, necrosis, was observed in medullated and non-medullated fibers of all sizes. The axons of fine fibers, however, underwent the process of degeneration more rapidly than those in coarse fibers and disintegration was complete earlier.

In reviewing the histologic evidence, it seems that axonal degeneration is initiated by the direct action of cold on living structures. The almost immediate appearance of changes following the injury and the progressive chain of events leading to complete destruction seems to negate any other explanation. That the early changes in axons were not due to ischemia was demonstrated by the morphologically normal appearance of axis cylinders which had been rendered ischemic for comparable periods. Furthermore, axonal damage was observed to commence much earlier than did any vascular changes which could be responsible for the outcome seen in similar experiments.[3,4] Moreover, another physical traumatizing agent, heat, is capable of producing identical changes in the axon cylinders.

The appearance of the morphologic changes resembles, within certain limits, those noted by Weddell and Glees[1] in wallerian degeneration. These authors reported a similar but retarded sequence of changes after section of the dorsal nerve trunk in rabbits' ears. The axis cylinders of non-medullated and fine medullated fibers were affected before those of coarse medullated fibers and their degeneration proceeded more rapidly. In general, axis cylinders of fine fibers were less resistant than those of coarse fibers. Blackwood,[5,6] using prolonged exposure to moderate temperature reduction (+4° C.), reported that all fibers were affected by cold. Fine non-medullated and medullated fibers, however, seemed to be more susceptible to cold than the others. On the other hand, Denny-Brown *et al.*[7] observed the axis cylinders of peripheral nerves to be selectively damaged by cold, the largest being the most sensitive and the smallest the least sensitive. Complete necrosis of all nerve structures has been observed within 24 hours after transient solidification of the exposed sciatic nerve of a cat. Solidification as brief as 10 seconds resulted in destruction of one third to one half of the total nerve fibers, but with escape of fine fibers. After cooling the nerve for 2 hours to a temperature between −4° and +3° C., the fine medullated fibers escaped destruction.

In the present investigation the final destruction involved axons of all fibers. The axis cylinders of fine medullated and non-medullated fibers, however, developed earlier and more rapid axolysis than those of coarse fibers. In the early stages after injury, more fine fibers were damaged than coarse fibers. This could indicate the high susceptibility to cold of axons in fine fibers as compared with those in coarse fibers. Apart from factors arising from differences in technique and tissues used, the present study does not explain why the results which we obtained differ from those of Denny-Brown and his co-workers.[7]

Since the fine fibers represent the nerve supply to the blood vessels and control the vasomotor functions, it would seem that the circulatory stasis following severe cold injury is, to a certain degree, the result of the progressive and complete degeneration of all nerve elements. Complete arrest of circulation after exposure of a rabbit's ear to severe cold has been observed, under similar conditions, as early as 10 minutes after thawing, although the flow in some channels persisted for a longer time.[8] Thus the stasis following a short period of reactionary hyperemia might be correlated with the almost immediate damage to the fine nerve fibers. Other factors, such as damage to the vascular wall and consequent escape of intravascular fluid associated with increase of tissue pressure constricting the vessels, increased resistance to flow, and intravascular clotting, will influence and contribute to the onset and extent of circulatory arrest.

Finally, it should be pointed out that the nerve fibers in all tissues, being very vulnerable to cold, may readily be damaged by this form of physical trauma.[9] The final outcome will depend, as in other tissues, on the degree and duration of reduction of temperature.[4-6,9] Since nervous tissue seems to be affected by cold more readily than the other tissues,[5,9] even the mildest degree of cold might result in transitory alteration in vasomotor function without causing any visible changes in other tissues.

Summary

The effects of severe cold (−20° to −30° C.) on the axis cylinders of peripheral nerve fibers in the skin of the dorsum of rabbits' ears were investigated. Intravital methylene blue staining was used. Degenerative changes in the axis cylinders were observed as early as 2 hours after cold injury. They consisted of fusiform swelling of axons with fibrillation, especially at the nodes of Ranvier. The final outcome was complete destruction of the axons. The degeneration of axons, as produced by severe cold, is apparently the result of the direct action of this physical agent. The axis cylinders of coarse medullated fibers seem to be more resistant to cold than those of non-medullated and fine medullated fibers.

REFERENCES

1. Weddell, G., and Glees, P. The early stages in the degeneration of cutaneous nerve fibres. *J. Anat.*, 1941–42, **76**, 65–93.
2. Pochin, E. E. Oedema following ischaemia in the rabbit's ear. *Clin. Sc.*, 1939–42, **4**, 341–347.
3. Lewis, R. B. Pathogenesis of muscle necrosis due to experimental local cold injury. *Am. J. M. Sc.*, 1951, **222**, 300–307.
4. Pirozynski, W. J., and Webster, D. R. Muscle tissue changes in experimental frostbite. *Ann. Surg.*, 1952, **136**, 993–998.
5. Blackwood, W. Injury from exposure to low temperature: pathology. *Brit. M. Bull.*, 1944, **2**, 138–141.
6. Blackwood, W., and Russell, H. Further experiments in the study of immersion foot. *Edinburgh M. J.*, 1945, **52**, 160–165.
7. Denny-Brown, D., Adams, R. D., Brenner, C., and Doherty, M. M. The pathology of injury to nerve induced by cold. *J. Neuropath. & Exper. Neurol.*, 1945, **4**, 305–323.
8. Crismon, J. M., and Fuhrman, F. A. Studies on gangrene following cold injury: VI. Capillary blood flow after cold injury, the effects of rapid warming, and sympathetic block. *J. Clin. Investigation*, 1947, **26**, 468–475.
9. Böttcher, H. Experimentelle Untersuchungen über örtliche Erfrierungen durch langdauernde Einwirkung geringer Kältegrade. *Virchows Arch. f. path. Anat.*, 1944, **312**, 464–485.

LEGENDS FOR FIGURES

FIG. 1. Normal non-medullated fiber with intact axis cylinder. × 475.

FIG. 2. Normal medullated fiber showing an intact axon with a node of Ranvier. The myelin sheath is faintly outlined. × 475.

FIG. 3. Fibers of normal nerve plexus with intact and disintegrating axons. × 450.

FIG. 4. Swelling of axis cylinder in medullated fiber and fibrillation at the node of Ranvier. Two hours after cold injury. × 450.

FIG. 5. Extensive swelling of axon and medullary sheath, which became sharply outlined. Two hours after exposure to cold. × 475.

FIG. 6. Swelling and fibrillation of axis cylinders in medullated fiber in nerve plexus; other fibers without signs of degeneration. Six hours after exposure to cold. × 450.

FIG. 7. Extensive swelling and fibrillation of axons in nerve plexus. Some axons are intact. Twelve hours after exposure to cold. × 450.

FIG. 8. Granular disintegration of axon in fine non-medullated fiber. Twelve hours after exposure to cold. × 475.

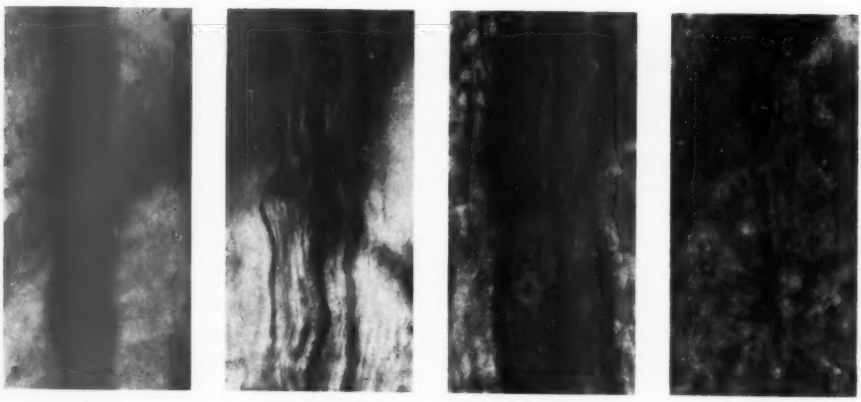
FIG. 9. Complete axolysis in coarse medullated fiber. Twenty-four hours after exposure to cold. × 475.

FIG. 10. Complete disintegration of axons in coarse medullated fibers. Seventy-two hours after exposure to cold. × 450.

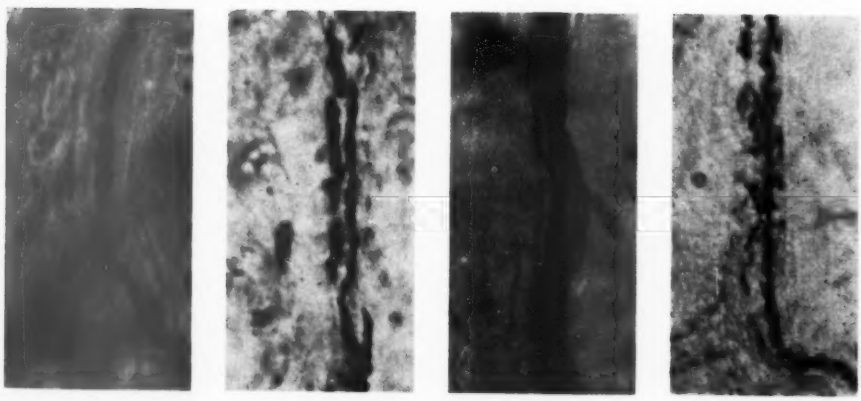
FIG. 11. Normal appearance of a medullated fiber. Twenty-four hours after 30 minutes of ischemia. × 475.

FIG. 12. Complete disintegration of axons in nerve plexus. Forty-eight hours after exposure to +65° C. for 4 minutes. × 425.

1 2 3 4



5 6 7 8



9 10 11 12

CHORIOEPITHELIOMA OF THE LUNG IN A FEMALE INFANT SEVEN MONTHS OLD *

SAUL KAY, M.D., and W. GLENN REED, M.D.

(From the Laboratory of Surgical Pathology and the Department of Pathology, Medical College of Virginia, Richmond 19, Va.)

Pick[1-3] was a staunch advocate of extrauterine chorioepitheliomas as early as 1905. A partial review of the literature yields reports of probable primary chorioepitheliomas of the ovary,[4] fallopian tube,[5] testicle, mediastinum,[6] retroperitoneum,[6] lung,[7,8] liver,[9] bladder,[10,11] jejunum,[12,13] and brain.[14]

The question of the origin of these growths is not easily settled. In the generative glands, such as the ovaries and testes, the theories of parthenogenesis and teratoma prevail.[4] In the teratomatous growths, it is believed that the trophoblastic elements eventually destroy all other tissues of the tumor, so that only chorioepithelioma remains. Extragenital tumors of the mediastinum and peritoneum are explained by displacement of germ tissue from the urogenital ridge.[6] In the bladder, teratomas are believed to arise from dysontogenetic rests of the dorsal mesodermal segments which are carried down to the bladder anlage by the developing wolffian duct.[10] Bostroem's theory[15] allows for the development of chorioepithelioma in widely scattered parts of the body. According to this theory, the tumor arises from undifferentiated germ cells ("serotinal wandering cells") which, irritated by humoral influences, react by proliferation to form the primary tumor cells. These cells develop into syncytial and Langhans' cells.

In the adult female, the question of a past pregnancy always arises. Since it is believed that a uterine chorioepithelioma may be completely extruded or devitalized with little or no trace of its presence, the possibility exists that the so-called primary ectopic tumors are in effect metastases. Even more significant is the fact that Schmorl (cited by Ceelen[16]) reported trophoblastic pulmonary emboli in women during pregnancy, particularly in difficult parturition, manual extraction of the placenta, placenta previa, eclampsia, and uterine tears. While Schmorl believed that tumor nodules could arise only from diseased trophoblasts, it was Pick's contention that even normal chorionic cells could give rise to chorioepithelioma. Relevant to the discussion is Lazarus and Schifrin's[17] report of a most unusual finding of multiple benign implants of chorionic villi involving the peritoneal cavity and the surface of the uterine adnexa.

* Received for publication, December 5, 1952.

Should pregnancy be positively excluded, as in virgins[18] or prepubertal patients,[4] most authors have assumed that the tumors arose in pre-existing teratomas, and that elements other than chorioepithelioma have been completely overgrown.

The following report is unique in that it concerns a case of ectopic chorioepithelioma in a 7-months-old infant. To the best of our knowledge, this is the earliest age ever reported for this extragenital tumor. In addition, we were able to make a thorough post-mortem study of all organs, since the infant died soon after pneumonectomy for what was thought to be a primary neoplasm of the lung.

Report of Case

The patient was a 7-months-old white female infant admitted to the Medical College of Virginia Hospital on May 12, 1952, for treatment of a tumor mass in the right chest. She had had a normal spontaneous delivery, and showed no unusual findings at birth. Three days after birth, however, a blood count revealed 2,280,000 red cells and 58 per cent hemoglobin. After several transfusions, the infant evidently remained well until about 2 months prior to admission. At that time, the patient developed fever, dyspnea, and anorexia which did not respond to penicillin. One month later, she began to cough, with the production of a blood-streaked sputum. Shortly thereafter she was found lying, after a coughing spell, in a pool of blood. Roentgenograms showed opacity of the entire right chest, with some clearing subsequently at the apex. Just before admission to this hospital, bright red blood was noted in the urine on three occasions.

On admission, the temperature was 100.4° F.; pulse, 150; and respirations, 34. The patient was fairly well developed, poorly nourished, in respiratory distress, and showed evidence of chronic illness. The chest was asymmetric with the right side more prominent than the left and showing only moderately lessened expansion with respiration. There was increased use of the accessory muscles of respiration. The right breast was larger than the left. The right chest was dull to percussion, and tactile and vocal fremitus were diminished. The left chest was clear. The remainder of the physical examination was non-contributary.

Laboratory findings showed hemoglobin to be 9.4 gm., with 3,430,000 red blood cells and 18,000 white blood cells per cmm., with normal differential white count. Roentgenograms confirmed the presence of a large mass filling most of the right chest. The mediastinum was displaced somewhat toward the left. By a lipiodol swallow a normal esophagus was shown, but there was moderate extrinsic pressure on the middle and lower portions of the esophagus, displacing it to the left.

Exploratory thoracotomy was done on the fourth hospital day. On the operating table, under a strong light, a thin growth of pubic hair was noted for the first time. On opening the chest, a large mass was encountered in the right lung, filling the thoracic cavity. It was adherent to the lateral chest wall, and bled copiously into the bronchial system upon manipulation. The central portion of the mass was necrotic. Only the upper lobe appeared to be aerated. A pneumonectomy seemed obligatory and was done without difficulty. Shortly after closure of the chest, the patient had cardiac standstill. The chest was reopened and cardiac rhythm restored by massage. After the second closure, the patient's condition seemed fairly good. She was returned to the ward but gradually declined. Despite all measures, death ensued approximately 4 hours after operation. Permission for necropsy was obtained.

PATHOLOGIC FINDINGS

Surgical Specimen

The surgical specimen (S-52 3316) was a right lung measuring 10 by 8 by 4 cm. and weighing 217 gm. The various lobes could be identified, but the bulk of the lower lobe appeared to be replaced by a hemorrhagic and necrotic tumor, approximately 5.5 by 4.5 cm. (Figs. 1 and 2). The lower lobe bronchus was stretched over the tumor and was displaced toward the hilar region. Within the upper lobe, two small tumor nodules, 0.4 by 1.2 cm. in diameter, each with a hemorrhagic rim, were identified. On cut section, the main mass consisted of hemorrhagic, spongioid, and necrotic tumor (Figs. 3 and 4).

Microscopically, the principal mass was a hemorrhagic neoplasm which had destroyed most of the lung parenchyma (Fig. 5). In general this tumor consisted of necrotic elements of varying size, arranged in irregular sheets and cords. These were set in masses of blood clot. In better preserved areas, sheets of swollen, faintly granular cells containing clear, giant-sized and bizarre nuclei were identified (Fig. 6). These were apparently Langhans' cells, and were often closely associated with syncytial elements containing similarly bizarre nuclei, but a more deeply staining and violescent cytoplasm (Figs. 6 and 7). Mitotic figures were fairly frequent and averaged 1 to 2 per high-power field. In some instances tumor encroached upon the bronchial mucosa of major bronchi and appeared partially to destroy the epithelium (Fig. 5). The nodules in the upper lobe consisted of similar neoplastic tissue and apparently represented metastases.

Diagnosis. Chorioepithelioma of lower lobe of right lung with metastases to right upper lobe.

Necropsy Findings

The body was that of a well developed 7-months-old female in a fair nutritional state. The slight increase in the size of the right breast and the presence of pubic hair, which were described before death, were not noted by the prosector. There was a right thoracic surgical incision, completely separating the sixth and seventh ribs. The right pleural cavity, from which the lung had been removed, contained about 50 cc. of bloody fluid. The mediastinum was approximately in the midline. The left pleural cavity was dry and free of adhesions, with the lung partially collapsed against the posterior wall.

The bronchial tree contained bloody, mucoid secretion. The left lung weighed 90 gm. There was atelectasis of the posterior and basilar

regions. On the undersurface of the upper lobe lay a subpleural, well circumscribed, spherical, hemorrhagic nodule, 0.2 cm. in diameter. The heart showed an area of diffuse hemorrhage within the myocardium at the base of the interventricular septum. This was believed to be due to trauma from cardiac massage prior to death. The liver weighed 400 gm. A second nodule, 0.3 cm. in diameter, but otherwise similar to that in the lung, was present on the inferior surface of the right lobe of the liver. The uterus and adnexa were infantile and grossly free of tumor. Other viscera were not remarkable.

The tissues were fixed in Bouin's fluid and stained routinely with hematoxylin and eosin. Periodic acid-Schiff's stain was used for the endometrium to demonstrate secretory granules of glycogen, and phosphotungstic acid hematoxylin was used for the pituitary gland in order to demonstrate basophilic granules. The ovaries were embedded together and cut completely into serial sections.

Microscopically, significant changes were noted in the liver, left lung, heart, ovaries, and endometrium. The metastatic nodules in the liver and left lung were composed of tumor similar to that described in the surgical specimen. The interventricular septum of the heart revealed diffuse hemorrhage causing compression and obliteration of muscle fibers without any inflammatory reaction. Serial sections of the ovaries failed to reveal the presence of a neoplasm. However, in each ovary (Figs. 8 and 9), cystic follicles were found in which the theca intima had undergone early luteinization. The endometrium was definitely thickened, and many of the glands revealed infranuclear and supranuclear vacuoles. Evidence for glycogen secretion, however, was minimal. The pituitary gland revealed hyperplasia of the basophilic cells, one of the features described in pituitary glands of patients dying with chorioepitheliomatous neoplasms.[19]

The cause of death was believed to be cardiovascular collapse brought on principally by the cardiac arrest following operation. The final diagnoses included: (1) chorioepithelioma of the lower lobe of the right lung with metastases to upper lobes of the right and left lung, and to liver; (2) myocardial hemorrhage and degeneration due to trauma (direct cardiac massage); (3) pulmonary edema; (4) theca-lutein cysts of ovary, bilateral; (5) hyperplasia of basophilic cells of pituitary gland.

Discussion

The problem of determining the genesis of this lesion meets with difficulties similar to those in the literature. One can obviously rule out a uterine neoplasm with secondary metastases to the liver and

lungs. We believe that the possibility of a primary ovarian tumor, whether it be teratoma or chorioepithelioma, has been fairly definitely excluded, since none was found after serial sectioning of all ovarian tissue. There remain only the possibilities of a primary teratoma of the lung, or emboli of chorionic villi from the mother to the child by way of the placental barrier.

The teratomatous theory has been amply championed in the literature[4,9,18] and perhaps this may be the best explanation for the present case. Such a presumption would force us to conclude that the right lung was the seat of a teratoma with eventual blotting out of all neoplastic elements save those of chorioepithelioma. The nodules in the left lung and liver are consequently metastases, since these are far too small to be considered primary growths.

Whether or not diseased or normal trophoblast from the mother can invade fetal blood vessels is difficult to prove. Unfortunately the question of functioning trophoblast in the mother of the patient was not investigated at the time of the birth of the child. An Aschheim-Zondek test performed on the mother 4 months after the death of the child was negative, but this may be of no significance. Suffice it to say that there are cases in the literature[20] supporting the transference of a maternal malignant neoplasm to the fetus. A provocative comment on this problem is found in Heilmann and Wappler's paper,[4] in which the authors quote a statement of Pick (page 1600) to the effect that it is possible that chorionic elements from the mother can be transported to an existing teratoma in the child. The teratomatous elements are destroyed because of the greater vitality of the trophoblast. (If Pick made the above statement, we have not been able to find it.) The transference of chorionic tissue from the mother to the fetus is an attractive theory in this case, because the distribution of tumors within the patient followed rather nicely the course of the umbilical vein through the liver, with ultimate dissemination to both lung fields.[21]

An interesting observation was the possible endocrine effect of this tumor. Should the cells of a chorioepithelioma be functioning, one could expect increased gonadotropic hormone in the blood. Clinically this was indicated in this patient by the enlarged right breast and the appearance of pubic hair. Unfortunately, the breast tissue was not examined histologically, so that definite proof is lacking. Histologically, there was indication of hormone effect on the ovaries, endometrium, and on the pituitary gland. Theca-lutein cysts are rather characteristic of chorioepitheliomas in general, and the endometrium

showed some evidence of secretory activity. Possibly the blood found in the urine may have been due to uterine bleeding.

Summary

A case of chorioepithelioma of the lung in a 7-months-old female infant is described. The tumor is believed to have been primary in the lung, since a complete necropsy failed to reveal the presence of any other possible primary. A nodule in the liver was believed to be too small for a primary tumor, but it may be of importance in the pathogenesis of the lesion as a whole. Other theories of origin of extrauterine chorioepithelioma have been considered inapplicable to this case.

An interesting finding was the discovery of endocrine effects, attributed to the tumor, on various parts of the body, including certain organs in which histologic changes were found.

We are indebted to Dr. Elizabeth Ferrington for translation of foreign publications.

REFERENCES

1. Pick, L. Zur Frage der Entstehung des Chorioepithelioms aus angeborener Anlage. *Virchows Arch. f. path. Anat.*, 1905, **180**, 172–179.
2. Pick, L. Zur Lehre vom Epithelioma chorioektodermale. *Zentralbl. f. Gynäk.*, 1905, **29**, 545–551.
3. Pick, L. Noch einmal zur Entstehung des Chorioepithelioms und Epithelioma chorioektodermale aus kongenitaler Anlage. *Zentralbl. f. Gynäk.*, 1905, **29**, 821–826.
4. Heilmann, P., and Wappler, R. Beitrag zur Frage des primären Chorionepithelioms der Keimdrüse. *Zentralbl. f. Gynäk.*, 1941, **65**, 1598–1601.
5. Solomons, B., and Smith, E. C. Primary chorion epithelioma of the fallopian tube. *J. Obst. & Gynaec. Brit. Emp.*, 1923, **30**, 162–171.
6. Hirsch, E. F. Extragenital choriocarcinoma, with comments on the male origin of trophoblastic tissues. *Arch. Path.*, 1949, **48**, 516–524.
7. Berman, L. Extragenital chorionepithelioma with report of a case. *Am. J. Cancer*, 1940, **38**, 23–31.
8. Gerber, I. E. Ectopic chorioepithelioma. *J. Mt. Sinai Hosp.*, 1935, **2**, 135–142.
9. De Zalka, E. Concerning ectopic chorionepithelioma. Report of two cases. *Am. J. Path.*, 1928, **4**, 59–73.
10. Hyman, A., and Leiter, H. E. Extratesticular chorioepithelioma in a male probably primary in the urinary bladder. *J. Mt. Sinai Hosp.*, 1943–44, **10**, 212–219.
11. Weinberg, T. Primary chorionepithelioma of the urinary bladder in a male. Report of a case. *Am. J. Path.*, 1939, **15**, 783–795.
12. Sears, J. B. Ectopic chorionepithelioma; report of case in which the lesion was situated in the jejunum. *Ann. Surg.*, 1933, **97**, 910–919.
13. Soresi, A. L. Chorionepithelioma of the jejunum. *Am. J. Cancer*, 1936, **28**, 583–586.
14. Stowell, R. E., Sachs, E., and Russell, W. O. Primary intracranial chorionepithelioma with metastases to the lungs. *Am. J. Path.*, 1945, **21**, 787–801.

15. Bostroem, E. Das Chorionepitheliom. Eine morphologische Studie. *Beitr. z. path. Anat. u. z. allg. Path.*, 1926–27, **76**, 293–373. (Cited by Sears.[12])
16. Ceelen, W. In: Henke, F., and Lubarsch, O. Handbuch der speziellen pathologischen Anatomie und Histologie. Julius Springer, Berlin, 1931, **3**, Pt. 3, 101–103.
17. Lazarus, J. A., and Schifrin, A. An unusual case of benign multiple chorionic villi implants in peritoneal cavity accompanied by hemoperitoneum. *Ann. Surg.*, 1942, **115**, 93–101.
18. Maizels, G. Probable chorionepithelioma in a virgin of seventy-one. *Lancet*, 1940, **1**, 690–692.
19. Mathieu, A., and Robertson, T. D. Teratomatous chorioepithelioma in a female and in a male; critical study of the literature for the years 1935, 1936, 1937; report of 2 cases in a male. *Internat. Abst. Surg. in Surg., Gynec. & Obst.*, 1939, **69**, 158–175.
20. Gray, J., Kenny, M., and Sharpey-Schafer, E. P. Metastasis of maternal tumour to products of gestation. *J. Obst. & Gynaec. Brit. Emp.*, 1939, **46**, 8–14.
21. Patten, B. M. Human Embryology. Blakiston Co., Philadelphia, 1946, pp. 686–687.

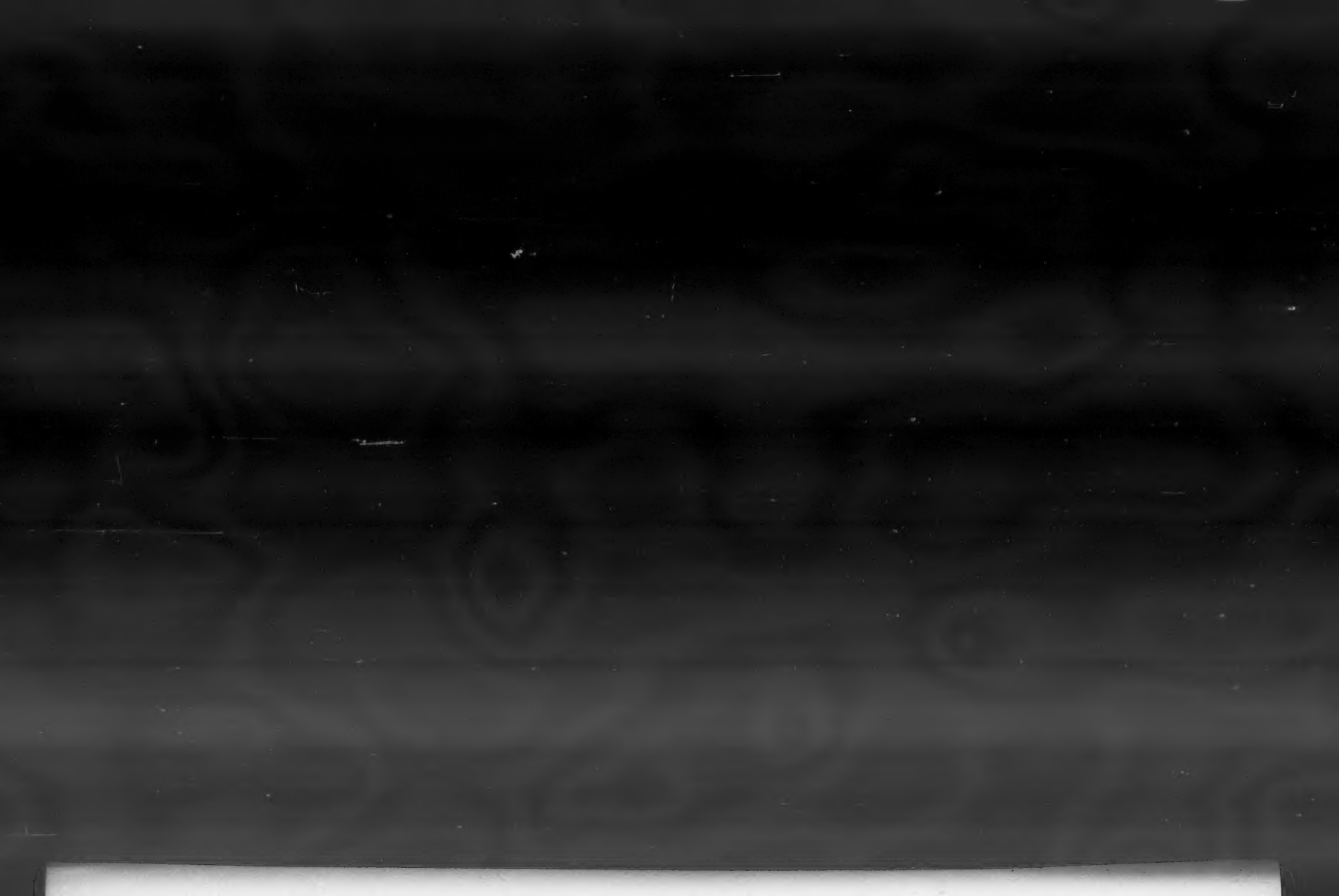
[*Illustrations follow*]

LEGENDS FOR FIGURES

FIGS. 1 and 2. Surgically removed right lung. The greater part of the lower lobe is replaced by neoplasm while the upper lobe is relatively uninvolved. A small subpleural metastatic nodule is shown at the right of each figure.

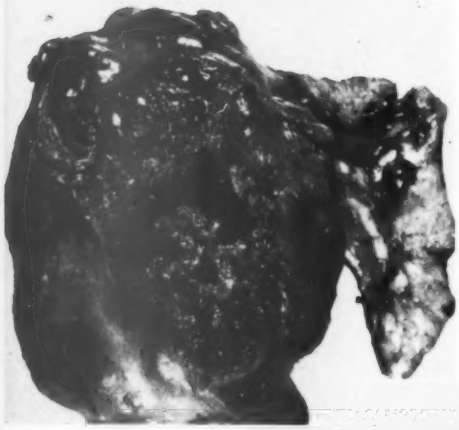
FIG. 3. The cut surface of the tumor is hemorrhagic. The lower portion of the figure shows a hemorrhagic metastatic nodule beneath the pleura.

FIG. 4. Another view of the cut surface of the tumor showing the characteristic hemorrhagic necrotic appearance of the interior of the neoplasm.



1

3



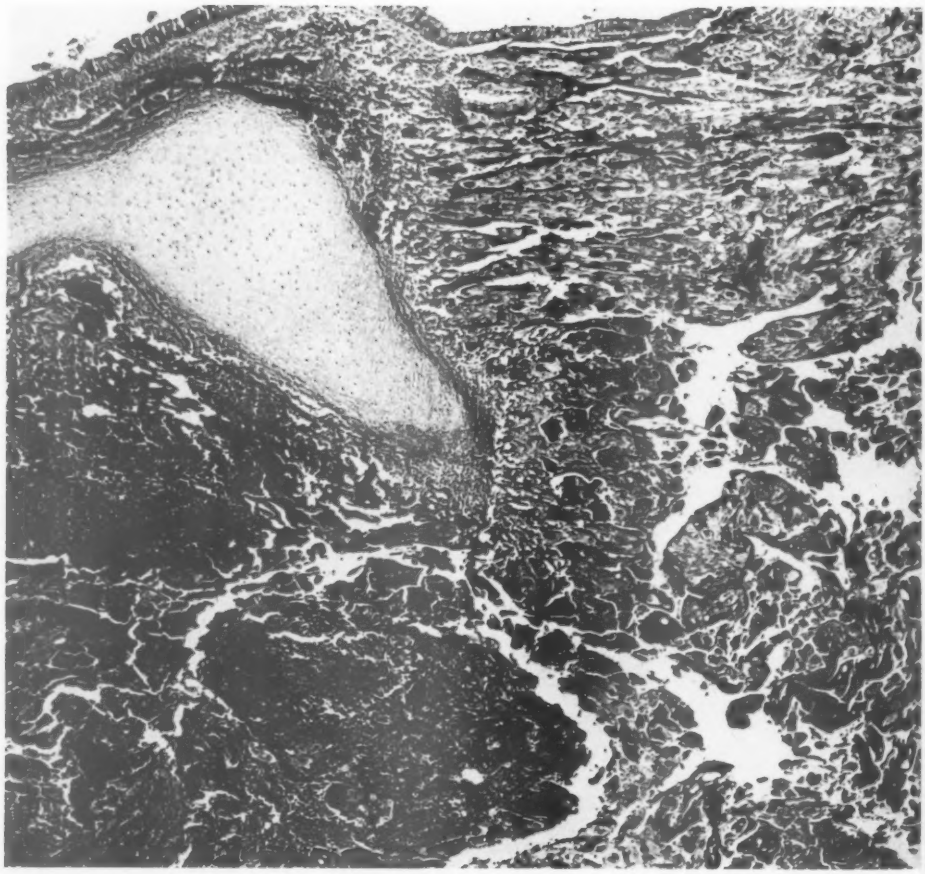
2

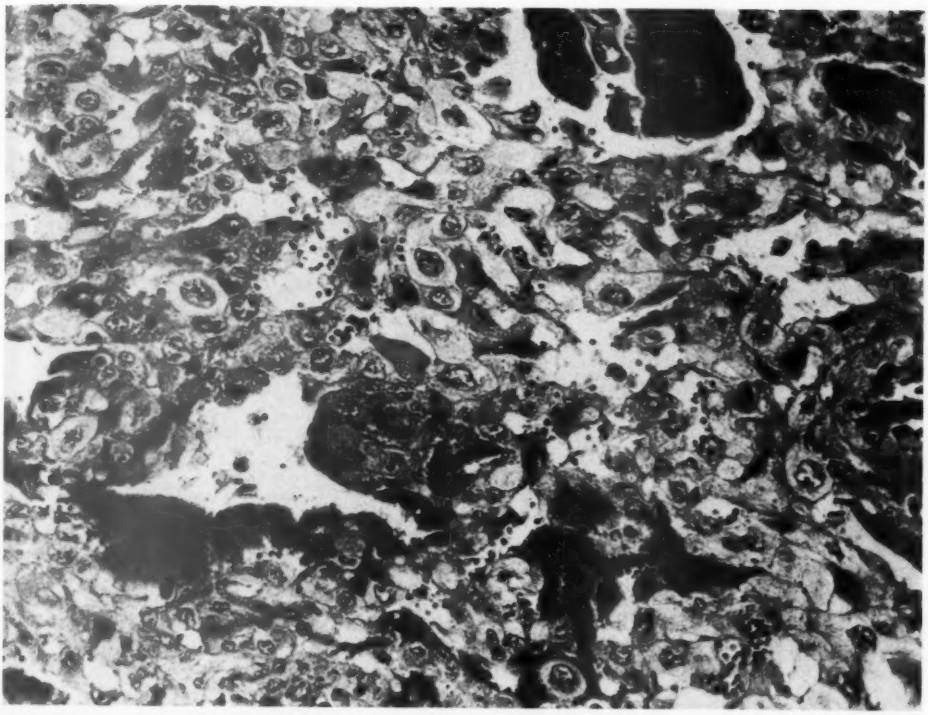
4

FIG. 5. Chorioepitheliomatous neoplasm surrounding bronchial cartilage and partially destroying mucous membrane. × 60.

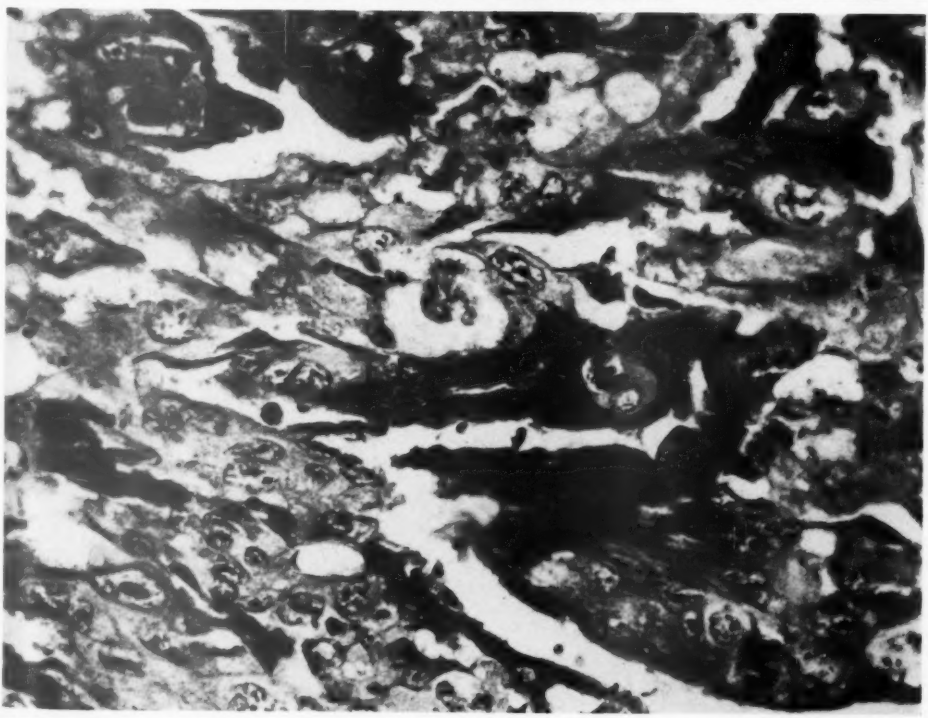
FIG. 6. Medium-power view of tumor showing Langhans' cells and scattered syncytial elements. × 150.

FIG. 7. High-power view showing greater details of tumor. × 400.



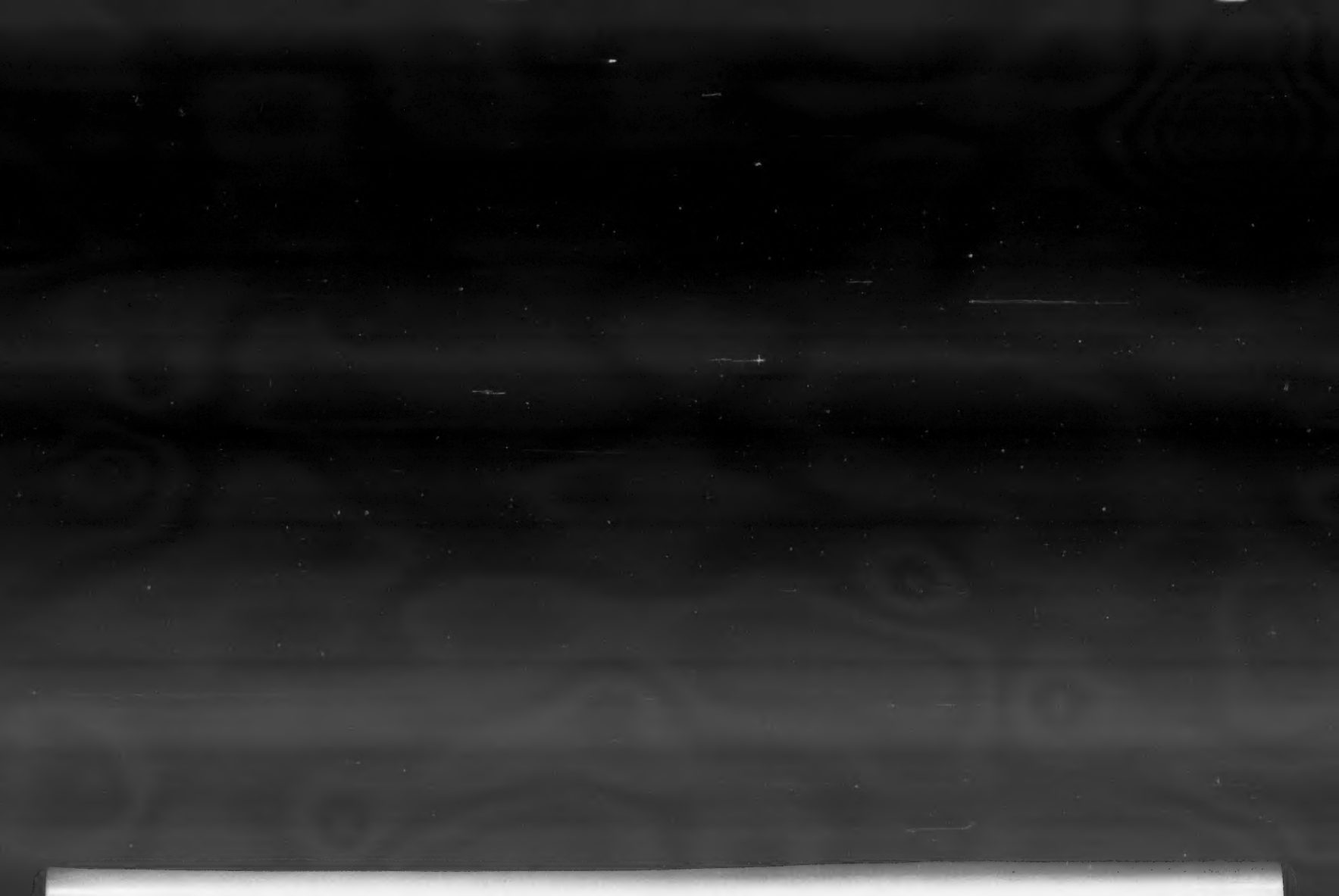


6

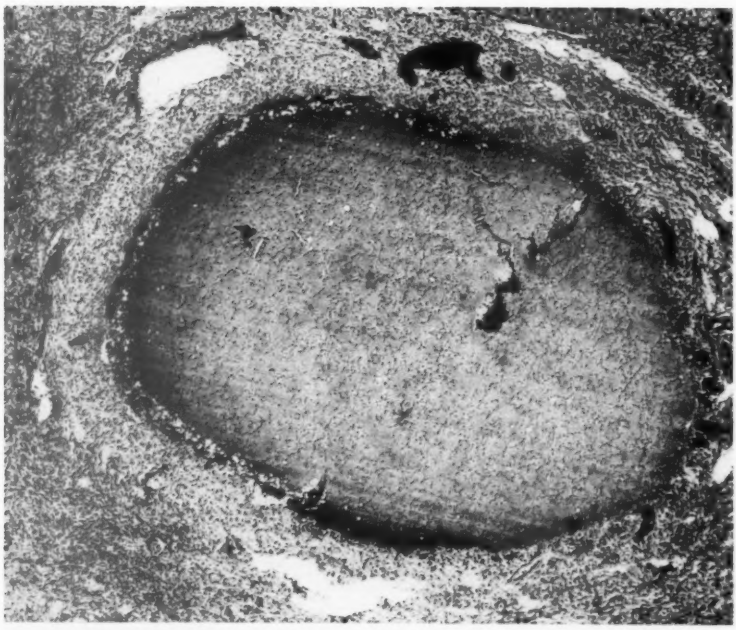


7

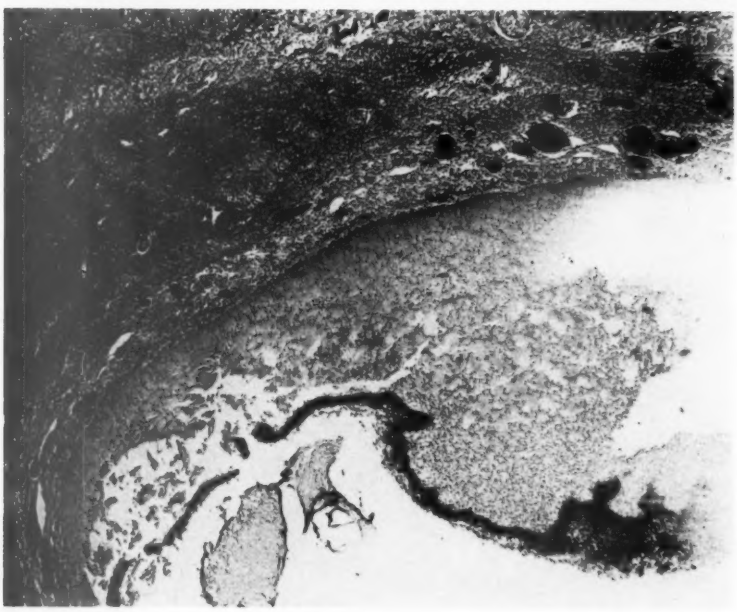
FIGS. 8 and 9. Theca-lutein cysts of right and left ovaries.



8



9



FIFTIETH ANNUAL MEETING
OF THE
AMERICAN ASSOCIATION OF PATHOLOGISTS
AND BACTERIOLOGISTS

ST. LOUIS, MISSOURI

APRIL 2ND, 3RD, AND 4TH, 1953

THE AMERICAN ASSOCIATION OF PATHOLOGISTS AND BACTERIOLOGISTS

Fiftieth Annual Meeting,
Jefferson Hotel,
St. Louis, Missouri
April 2nd, 3rd, and 4th, 1953

PRESIDENT FELDMAN IN THE CHAIR

BUSINESS MEETING

April 2, 1953

Upon nomination of the Council, the Association elected the following officers:

President	DR. JAMES B. MCNAUGHT
Vice-President	DR. G. LYMAN DUFF
Secretary	DR. ALAN R. MORITZ
**Treasurer*	GEN. ELBERT DECOURSEY
Incoming Member of Council	DR. SIDNEY FARBER

The President announced that the following non-voting officers had been elected by the Council:

Assistant Secretary	DR. J. LOWELL ORBISON
**Assistant Treasurer*	DR. ELSON B. HELWIG

For the Council, the President announced the following actions:

Election of New Members

Lester Adelson, Cleveland, Ohio
Wilhelm S. Albrink, New Haven, Conn.
Raymond Bangle, Jr., Bethesda, Md.
Earl P. Benditt, Chicago, Ill.
Edward J. Benz, Bethlehem, Pa.
James I. Berkman, New York, N.Y.
Guillermo M. Carrera, New Orleans, La.
Fred C. Collier, Winston-Salem, N.C.
Isaac Costero, Mexico, D.F.
Gyula I. DeSuto-Nagy, New Haven, Conn.
Theodore Ehrenreich, New York, N.Y.
Harlan I. Firminger, Kansas City, Kans.
Charles F. Geschickter, Washington, D.C.
Helen A. Horn, Bethesda, Md.
William E. Jaques, Newbury, Mass.
Chester K. Jones, Rochester, N.Y.

* Effective January 1, 1954.

Saul Kay, Richmond, Va.
G. F. Kipkie, Kingston, Ontario
Jerome Kleinerman, Shaker Heights, Ohio
H. Lee Large, Jr., Charlotte, N.C.
Richard G. McManus, Pittsburgh, Pa.
Berne L. Newton, Houston, Texas
H. Preston Price, Ridgewood, N.J.
Robert W. Prichard, Winston-Salem, N.C.
Ronald C. Sniffen, Worcester, Mass.
Leland D. Stoddard, Prairie Village, Kans.
Edgar B. Taft, Cambridge, Mass.
Arthur C. Upton, Oak Ridge, Tenn.
Helenor Campbell Wilder, Washington, D.C.

Acceptance, with regret, of the resignations of Cornelia M. Downs, John E. Kraus, David Marine, Grace M. Sickles, and Holland N. Stevenson.

With deep regret, the recording of the deaths of Zera E. Bolin, Eugene Case, William J. Elser, Marshall Fabyan, Hugo A. Freund, Raymond A. Kelser, William deB. MacNider, William Magner, and Douglas Symmers.

The re-election of Dr. R. Philip Custer to the Editorial Board of *The American Journal of Pathology* for a period of six years.

The President announced that the next annual meeting of the Association will be held in Philadelphia, Pennsylvania, on April 8, 9, and 10, 1954. Attention of the members is called to the fact that this date falls in the week preceding Good Friday. The topic for the symposium is "Diseases of the Nervous System and of the Neuromuscular Apparatus."

The President further announced that the annual meeting in 1955 would be held in Houston, Texas; the dates to be announced later.

* * * * *

Note: Through an oversight, the following information was not included in the business meeting of the Association. During the year 1952–53 a questionnaire was circulated to learn how many of the members of the Association would be interested in a group plan insurance in the event that such would be sponsored by the Association. In a total membership of 850, there were 590 postcard replies, the majority of which indicated interest in such a plan. The matter was brought to the attention of the Council at its meeting, April 1, 1953, and after full consideration the Council decided that sponsorship of a group plan insurance would be without the scope of the policy of the Association, and it was voted to take no action in the matter of group insurance.

Alan R. Moritz, *Secretary*

REPORT OF THE TREASURER

The report of the Treasurer was submitted to the Council and accepted. It was accompanied by a letter of certification from Frank D. Flynn, Auditor, Melrose, Massachusetts. In condensed form, the Treasurer's report follows:

Checking General Account

Receipts

Balance on hand, January 1, 1952		$ 5,701.92
Membership dues	$ 9,619.16	
Interest on bonds	500.00	
		10,119.16
		$15,821.08

Disbursements

American Journal of Pathology	$ 8,512.00	
Membership lists	595.65	
Officers' expenses at meetings (printing, badges, travel, etc.)	659.01	
Secretary's office, clerical	$350.00	
Typing membership lists	90.35	
Office expenses	277.27	
	717.62	
Treasurer's office, clerical	$150.00	
Auditor	35.00	
Printing, safety deposit, etc.	21.40	
	206.40	
		10,690.68
Balance on hand, December 31, 1952		$ 5,130.40

Investment Account

Balance, January 1, 1952		$34,000.00
Interest, savings banks		
Provident Institution for Savings	$ 56.38	
Franklin Savings Bank	56.38	
Cambridge Savings Bank	193.02	
National Shawmut Bank	20.05	
		325.83
Balance, December 31, 1952		$34,325.83

Inventory

U.S. bonds, series G	$20,000.00
Provident Institution for Savings	4,056.38
Franklin Savings Bank	4,056.38
Cambridge Savings Bank	4,193.02
National Shawmut Bank	2,020.05
Total, December 31, 1952	$34,325.83

Sidney Farber, *Treasurer*

SCIENTIFIC PROCEEDINGS

FAT EMBOLI IN LUMINA OF RENAL ARTERIOLES AND GLOMERULAR CAPILLARIES OF DIABETIC PATIENTS WITH KIMMELSTIEL-WILSON LESIONS.* W. Stanley Hartroft, Department of Pathology and Banting and Best Department of Medical Research, University of Toronto, Toronto, Canada.

Abstract. The author has previously reported the presence of fat emboli in renal arterioles and glomerular capillaries of rats subjected to prolonged periods of dietary choline deficiency. This was observed to produce focal glomerular lesions which closely resembled some forms of Kimmelstiel-Wilson lesions encountered in diabetic patients. Wilens, S. L., Elster, S. K., and Baker, J. P. (*Ann. Int. Med.*, 1951, **34**, 592–607) have described a significant incidence and degree of glomerular lipidosis in intercapillary glomerulosclerosis in man. In a series of frozen sections of kidneys obtained at necropsy from diabetic patients showing this lesion, the findings of Wilens *et al.* have been confirmed, and in addition, fat emboli were found within lumina of arterioles, glomerular capillaries, and post-glomerular vessels. This reinforces the conclusion suggested by the evidence obtained from the study of the pathogenesis of the renal lesions in choline-deficient rats, that a chronic form of intermittent fat embolism may exist, without a history of trauma, leading to focal glomerular lesions not unlike the Kimmelstiel-Wilson type. It is not suggested on this evidence that choline deficiency *per se* plays any direct rôle in the production of the glomerular lesions of diabetic man, but there are indications that some of the pathologic glomerular changes in the animals studied and in diabetic humans may have a common pathogenesis. It will be most important to determine if the fatty liver of insulin deficiency (*i.e.*, of diabetes) in animals can give rise to intermittent fat embolism and glomerular lesions.

MORPHOLOGIC AND FUNCTIONAL ANALYSIS OF THE CANINE LUNG AFTER PULMONARY VEIN DIVISION. John P. Wyatt, Donald Burke, and J. Mudd (all by invitation), St. Louis University School of Medicine, St. Louis, Mo.

Abstract. The problem of pulmonary congestion in experimental dogs was studied by division and ligation of the pulmonary veins from the right or left lung. No deaths occurred after ligation of the left pulmonary veins, but the mortality was high with occlusion of the pulmonary veins on the right side. This high mortality following right pulmonary ligation was obviated with postoperative administration of penicillin. Observations conducted over a period of 1 year on the structural alterations, directional spread of the pulmonary congestion, and ultimate resolution were integrated with sequential functional analyses. Following the congestive state, the lung progressively cleared and at the end of 6 months there was little or no morphologic evidence of damage to the lung. Lack of permanent changes in the lungs following pulmonary ligation indicates that operative interference with the pulmonary vein outflow does not lead to devitalization. Functional studies following recovery from pulmonary vein ligation suggest that these lungs were unable to carry on significant respiratory exchange of gases.

STUDIES OF THE VENOUS COLLATERAL CIRCULATION OF THE LUNG. Alfred Hurwitz (by invitation), Averill A. Liebow, Paul Kunkel (by invitation), and Ronald W. Cooke (by invitation), Newington Veterans Administration Hospital, Newington, Conn., and Yale University School of Medicine, New Haven, Conn.

Abstract. After ligating the major pulmonary veins close to the auricle in dogs,

* This work was supported in part by a grant from the National Research Council of Canada.

infarction of the lung does not occur. As in previously reported observations in man, the bronchial veins of the dog are joined by short broad channels to the largest pulmonary veins and permit of a sufficient drainage. Within 3 months a very extensive venous collateral circulation is found to deliver the blood from the lung on the side of ligation to the *right* auricle. It is apparent from the study of bronchovascular casts that some of the collateral vessels communicating with the pulmonary veins at the hilum represent an expansion of the bronchopulmonary venous system. Others are obviously newly formed vessels, since they enter via adhesions, on the mediastinal aspect largely from the internal mammary veins (superior caval system), and on the lateral aspect from the intercostal veins (azygous system). Many collateral vessels of the last two groups are remarkable in that they join the distal ends of pre-existing pulmonary veins, end-on. The mechanism for establishing these connections is unknown, and its elucidation would contribute to the solution of some of the fundamental problems of the development of collateral circulation in general.

Correlated functional observations in these animals have indicated also a striking rise in the oxygen content of the azygos vein, and a flow through the venous collateral channels within 3 months' time of 1/6 to 1/5 of the expected flow in the intact lung.

One application of this work may be in the treatment of congenital transposition of the great vessels, in which ligation of the pulmonary veins of one lung should create a left-to-right shunt, useful in carrying oxygenated blood to the right heart and transposed aorta.

THE PERMEABILITY OF LUNG PARENCHYMA TO PARTICULATE MATTER. Paul Gross and Marian L. Westrick (by invitation), the Industrial Hygiene Foundation, Mellon Institute, Pittsburgh, Pa.

Abstract. Excised rat lungs were made to respire air heavily charged with carbon dust. In addition, India ink was injected intratracheally into living rats. Sections of the excised lungs after varying periods of artificial respiration and sections of the lungs from rats sacrificed at different intervals following intratracheal injection showed penetration of extracellular pigment into the lung stroma and its migration toward bronchi and vessels. It is concluded from these experiments that particulate matter may penetrate the respiratory membrane without the mediation of phagocytic cells. Once this penetration has been accomplished, pigment may migrate, without the aid of cells, toward peribronchial and perivascular lymphatics. Penetration of the respiratory membrane occurs in multiple scattered foci which are conditioned by alterations in the character of the membrane such as take place in inflammation. Physical factors which make penetration of the respiratory membrane possible are the fluctuating pressures of the respiratory cycle and the respiratory excursions of the lung tissue.

THE PATHOLOGY OF THE PULMONARY ARTERIOLES IN MITRAL STENOSIS COMPLICATED BY GIANT LEFT AURICLE. Jerome Kleinerman (by invitation), Cleveland City Hospital and Western Reserve University, Cleveland, Ohio.

Abstract. The pathologic changes in the intrapulmonary arterioles of 7 cases of mitral stenosis complicated by giant left auricle have been compared with the arteriolar structure as seen in mitral stenosis without giant left auricle, and with the pulmonary arterioles of normal individuals dying acutely. The working hypothesis which prompted this study was that a tremendous left auricular reservoir might act as a buffer and thus spare the intrapulmonary vessels. The control groups were selected so that their mean ages corresponded closely to that of a giant left auricle group. In addition, in the control group of mitral stenosis alone, severe degrees of stenosis were selected corresponding to the degree of mitral disease in the giant left auricle group. The duration of disease was difficult to correlate since this was often indefinite. The arterioles were studied quantitatively according to the method of

Kernohan, Anderson, and Keith. Sections stained by Masson's trichrome and elastic tissue methods also were studied.

Results suggest that in mitral stenosis associated with giant left auricle, the pulmonary arterioles show a slightly greater quantitative reduction in caliber of their lumina than do the pulmonary arterioles in mitral stenosis without giant left auricle. The giant left auricle therefore appears to have no ameliorating effect on the pulmonary arteriolar changes in mitral stenosis.

LESIONS IN SURGICALLY REMOVED AURICULAR APPENDAGES AND THEIR SIGNIFICANCE. A. Edwards (by invitation), John Denst, and Karl T. Neubuerger, General Rose Memorial Hospital, Denver, Colo.

Abstract. The histologic findings in 50 auricular appendages removed during valvulotomy were demonstrated. The incidence and significance of non-specific myocarditis, Aschoff nodules, endocardial and myocardial fibrosis, thrombosis, and thrombus organization was discussed. An attempt at correlation of the pathologic changes with the postoperative condition of the patient was made.

THE VASCULARITY OF THE EARLY SUBCUTANEOUS NODULE OF RHEUMATOID ARTHRITIS. Leon Sokoloff, J. J. Bunim (by invitation), and R. T. McCluskey (by invitation), New York University College of Medicine, New York, N.Y., and National Institute of Arthritis and Metabolic Diseases, Bethesda, Md.

Abstract. The mature subcutaneous nodule of rheumatoid arthritis is an indolent lesion in which large amounts of necrotic detritus and scar tissue are the dominant components. In the early stages of its development, however, the nodule is a highly vascular lesion. Seven cases are presented to illustrate four principal features of interest concerning these vessels:

1. Proliferation of vascular granulation tissue appears to be an early and integral feature of the development of the nodule.

2. In several instances it has been possible to demonstrate that the characteristic processes of necrosis and the formation of palisades of radially arranged, elongated cells occur about preformed as well as newly proliferated vessels. The necrosis at the periphery of the granulation tissue suggests that the process is a centrifugal one with respect to the vessels. The fact that the process of necrosis apparently follows the planes of the connective tissue about the vessels suggests that the necrosis-producing agent is fluid-borne into them from the vessels.

3. Inflammatory changes and necrosis may be seen within the vessels in these nodules and in the adjacent subcutaneous tissue.

4. In several instances the vascular lesions in the nodules occurred in individuals in whom it was possible to demonstrate the presence of similar arteritic lesions in striated muscle and synovial membrane.

These findings suggest that the blood vessels play a special rôle in the pathogenesis of the subcutaneous nodule. Furthermore, they indicate that the occurrence of vascular lesions in the nodules is a local manifestation of a more generalized, specific, rheumatoid arteritis.

RENAL LESIONS IN SCLERODERMA. Richard L. Swarm (by invitation) and Frederick G. Germuth, Jr., Washington University School of Medicine, St. Louis, Mo.

Abstract. Renal insufficiency may be an important cause of death in scleroderma. Marked renal lesions were found in 4 of 6 cases of scleroderma in which necropsy was performed. The terminal clinical courses in 3 cases were identical, consisting of rapidly developing oliguria, anuria, and uremia. The lesions in the kidneys of these patients were similar. In the gross, numerous small infarcts were present in the cortex. Microscopically, there was intimal proliferation and "hyaline" alteration of the interlobular arteries, and "hyaline" alteration of the walls of the arterioles

and of the glomerular loops. Similar changes in the arterioles were observed in the glomerular zone of the adrenal gland and in the pancreas and were associated with recent miliary infarcts. In a fourth patient dying in uremia, the kidneys showed a marked atypical subacute glomerulonephritis. A fifth case without renal symptoms contained granulomatous lesions about and involving the arteries within the kidney and liver, compatible with polyarteritis nodosa. The renal changes in scleroderma are varied. However, the alterations observed in the group of 3 cases were similar and seem to represent a pattern distinctive for scleroderma.

FIBROSIS AND HEMOSIDEROSIS OF THE LIVER AND PANCREAS IN NINE PATIENTS WITH COOLEY'S ANEMIA. John T. Ellis, Carl H. Smith (by invitation), and Irving Schulman (by invitation), the New York Hospital—Cornell Medical Center, New York, N.Y.

Abstract. Eleven cases of Cooley's anemia have been followed clinically for long periods of time and also studied anatomically. Fibrosis of the liver and pancreas, together with extensive hemosiderosis of these and other organs, was present in the 4 cases examined post mortem after periods of clinical observation ranging from 6 to 20 years; while fibrosis and hemosiderosis were found in the biopsied livers of 5 remaining cases which had been under observation for periods of 6 to 15 years. In 2 additional patients who died at 2 and 4 years of age respectively, visceral fibrosis was entirely absent, while hemosiderin was found in the liver but not in the pancreas in each instance. These findings bear upon the relationships of iron deposition to visceral fibrosis, and they have special interest in relation to the observation that fibrosis of the liver and pancreas was not found, though hemosiderosis was extensive, in 4 cases of Cooley's anemia in infants and young children previously studied post mortem by Whipple, G. H., and Bradford, W. L. (*J. Pediat.*, 1936, 9, 279–311).

In the 9 cases with visceral fibrosis mentioned above, the hepatic changes comprised a finely nodular cirrhosis. The changes in the pancreas consisted of slight to marked interlobular and intralobular fibrosis in 4 cases. The degree of visceral hemosiderosis, as graded histologically, could not be correlated with the amount of transfused blood which varied from 1000 to 102,000 cc. (containing approximately 0.5 to 51 gm. of iron). Furthermore, chemical analysis of a sample of liver from one case indicated that the whole organ contained 30 gm. of iron, whereas the patient had received only 6.6 gm. of iron from multiple transfusions.

It seems noteworthy that the 2 youngest patients in the series (aged 2 and 4 years respectively) failed to manifest fibrosis of the liver or pancreas, while the 2 oldest ones (aged 20 and 27 years respectively) had the most advanced degrees of hepatic and pancreatic fibrosis seen in the entire group. Furthermore, one of the older patients (aged 20) developed diabetes mellitus during the last few weeks of life. These observations suggest that hepatic and pancreatic fibrosis may have developed quite slowly in the cases here reported, becoming evident only after the life span had been lengthened by therapy.

PATHOLOGIC CHANGES IN HUMAN STARVATION. Conrad L. Pirani, University of Illinois College of Medicine, Chicago, Ill., Robert C. Stepto (by invitation), Army Medical Nutrition Laboratory, Chicago, Ill., and Earl B. Wert (by invitation), Mobile, Ala.

Abstract. The pathologic changes of chronic starvation were studied in 59 cases from the Dachau concentration camp in the files of the Armed Forces Institute of Pathology. The caloric intake of these patients for many months ranged between 1000 and 250 calories per day. Their average age was 35.5 years, their height 163 cm., and their weight 43 kg., corresponding to a loss of approximately 35 per cent of the presumed normal body weight. The main changes attributable to starvation were atrophy of muscular and adipose tissues, hyperkeratosis and pigmentation of the

skin with atrophy of the sebaceous glands, thickening of the stratified squamous epithelium of the tongue with pseudo-epitheliomatous hyperplasia, brown atrophy of the heart with a high incidence of serous myocarditis, poor tissue reaction in cases of pulmonary tuberculosis with predominance of exudative forms, necrotizing character of bacterial pneumonia, brown atrophy of the liver with moderate fatty changes, hemosiderosis and reticulo-endothelial hyperplasia of liver and spleen, atrophy of lymphatic tissue, cystic dilatation of pancreatic acini, atrophy of the adrenal glands with depletion of cortical lipids, and, finally, atrophy of the thyroid gland with depletion of colloid. The absence of clear-cut signs and changes of specific vitamin deficiencies was emphasized.

VISCERAL LARVA MIGRANS IN MAN CAUSED BY THE DOG ASCARID TOXOCARA CANIS. G. M. Carrera (by invitation) and John H. Dent (by invitation), Tulane School of Medicine, New Orleans, La.

Abstract. Sporadic cases of unexplained eosinophilia, often observed in children, have bewildered clinicians and pathologists for a long time. Various names, such as Loeffler's syndrome, familial eosinophilia, Weingarten's disease, and others, have been given to entities characterized by eosinophilia of obscure etiology and variable clinical manifestations. In many of these cases no parasites are found in the stools. We have studied in serial sections liver obtained for biopsy from 5 children with eosinophilia, low-grade fever, malaise, hepatomegaly, and signs of pulmonary involvement. Granulomatous lesions, consisting of focal areas of necrosis surrounded by epithelioid and giant cells and infiltrated with eosinophils and other inflammatory cells, have been observed in all these specimens. In 3 cases larval nematodes were found in the lesions and were identified by one of our collaborators, Dr. Paul C. Beaver, as Toxocara, probably *Toxocara canis*, the dog ascarid. Thus, it appears that larvae of certain nematodes considered as specific parasites of lower animals possess the ability to invade the tissues of the human host, and can produce substantial injury. There is evidence that these larvae are incapable of completing their life cycle and thus migrate continually through the various organs until they eventually perish. Such larvae may live in the tissues for months and, since they do not complete their life cycle and take residence in the intestine as adult worms, no parasites or ova appear in the stools.

Visceral lesions may be produced, of course, by the larvae of helminths such as *Ascaris lumbricoides* whose definitive host is man. However, the demonstration that human disease can be produced by parasites of lower animals and that the larvae of these parasites may survive for long periods and produce sustained eosinophilia and other systemic manifestations without being capable of completing their cycle suggests the possibility of a common etiologic basis for a whole group of syndromes characterized by unexplained eosinophilia. The facts that domestic animals, especially dogs, are almost universally parasitized and live in such intimate relationship with man, together with the frequency of unexplained eosinophilia and the finding of dog ascarid larvae in some of the cases we have studied, suggest that the condition may be of fairly common occurrence.

A CLINICOPATHOLOGIC STUDY OF "MIKULICZ'S DISEASE."* Winfield S. Morgan (by invitation) and Benjamin Castleman, Massachusetts General Hospital, Boston, Mass.

Abstract. The results of a study of 18 cases of "Mikulicz's disease" were presented. In the typical case the disease develops clinically as an asymptomatic enlargement of one or more salivary or lacrymal glands, and occurs most frequently in middle-aged women. Grossly, the diseased gland is characterized by enlargement of indi-

* See also this issue, pp. 471–503.

vidual lobules with preservation of the normal lobular architecture. Microscopically, it is characterized by a gradual replacement of glandular parenchyma by lymphoid tissue, and an intra-ductal proliferation of epithelial and myoepithelial cells leading to the formation of epi-myoepithelial islands. A study of 10 control cases of malignant lymphoma of the salivary gland, proved by lymph node biopsy or necropsy, indicated that the presence of the epi-myoepithelial islands is the most dependable means of differentiating Mikulicz's disease from malignant lymphoma. The etiology is not established; however, evidence was presented suggesting that Mikulicz's disease is not a distinct entity but rather one part of the group of diseases related to rheumatoid arthritis including Sjögren's syndrome (keratoconjunctivitis sicca, rhinostomato-pharyngo-laryngitis sicca, swelling of the parotid glands, and chronic polyarthritis).

SYSTEMIC NON-SUPPURATIVE PANNICULITIS. Bernhard Steinberg, Toledo Hospital Institute of Medical Research, Toledo, Ohio.

Abstract. Nodular panniculitis (Weber-Christian disease) has been described previously. There are approximately 48 cases in the literature. However, in addition to the involvement of the subcutaneous tissue there is also a systemic condition which has not yet been described. The lesions are of two types. There is a panniculitis surrounding several of the viscera. In addition, there are changes within the parenchyma of some of the organs, especially the liver. The nature of these changes and the clinical correlations were described.

THE SELECTIVE DESTRUCTION OF THE ALPHA CELLS BY COBALTOUS CHLORIDE AND ITS PHYSIOLOGIC IMPLICATIONS. Bruno W. Volk, Martin G. Goldner (by invitation), and Sydney S. Lazarus (by invitation), Jewish Sanitarium and Hospital for Chronic Diseases, Brooklyn, N.Y.

Abstract. The origin and the physiologic rôle of the hyperglycemic factor (HGF or glucagon) are subjects of considerable controversy. Some experimental evidence seems to indicate that this factor is secreted by the alpha cells of the pancreatic islets. The discovery that cobaltous chloride causes selective damage or destruction of these cells in various animal species has opened a new approach to this problem. The alpha cell damage is observed within 1 hour after the intravenous administration of $CoCl_2$ and persists for several days. The administration of $CoCl_2$ is also accompanied by an immediate and significant rise of the blood sugar level which may persist for from 2 to 4 hours. This is followed by a gradual return to pretreatment levels. Occasionally a very short-lasting and mild hypoglycemic phase is observed preceding the return to normal. Despite the persistent alpha cell damage normoglycemia is then maintained. Moreover, during this period of demonstrable alpha cell damage new hyperglycemic episodes can be elicited in the same animal by repeated injections of $CoCl_2$ at brief intervals.

These observations may be interpreted by two alternative hypotheses: (A) $CoCl_2$ has two independent actions. On the one hand, it acts as a hepatic irritant causing glycogenolysis; on the other, it injures the alpha cells. (B) The damage to the alpha cells liberates HGF which raises the blood sugar level by hepatic glycogenolysis. Support for the former hypothesis is derived from observations on depancreatized dogs in whom the administration of cobalt raises the blood sugar level similarly to that observed in the intact animal, in spite of the absence of the pancreas. Although in alloxanized animals temporary fluctuations of the blood sugar may occur immediately after cobaltous chloride administration, there is no permanent effect upon the diabetic status of these animals. Since destruction of the alpha cells caused no permanent effect on the blood sugar level in either normal or alloxanized animals, it must be presumed that their physiologic rôle in blood sugar homeostasis is negligible. Hepatocellular damage after cobalt administration has not been observed, but marked

depletion of the hepatic glycogen content is seen. Other studies utilizing the selective action of cobaltous chloride are in progress to evaluate further the physiologic function of the alpha cells.

MYOCARDIAL HEMORRHAGES PRODUCED BY GLYCOL ESTERS. F. W. Hartman and V. G. Behrmann (by invitation), Henry Ford Hospital, Detroit, Mich.

Abstract. Localized myocardial hemorrhages, principally in the subendocardial area, were observed in the course of the experimental use of glycol esters administered intravenously in 11 dogs as a replacement for blood withdrawn. The total quantity of ester injected ranged from 1.2 gm./kg. to 3 gm./kg. Studies on the physiologic mechanism and pathology of these hemorrhages were presented.

THE SIGNIFICANCE OF THE STOMACH IN IODINE METABOLISM. Rafael Dominguez and William C. Schmidt (by invitation), St. Luke's Hospital, Cleveland, Ohio.

Abstract. The purpose of this study was to determine the rate at which radioactive iodine is removed from the blood stream following a single rapid intravenous injection of I^{131}. Dogs were used under nembutal anesthesia and fully heparinized. The blood from an artery, usually the femoral, was made to flow into a jacketed G.M. counting tube and back to a vein, usually the femoral on the same side. The urine was collected by catheter for approximately hourly intervals in all experiments. The changes in the activity of circulating blood were recorded continuously and automatically for 3 to 4 hours from the beginning of the injection. The activity of the thyroid gland was determined in some experiments, either by counting *in vivo* or by wet-ashing the thyroid gland after thyroidectomy. In some instances, the I^{131} content of the stomach was determined in the gastric juice obtained by stomach tube, with and without rinsing of the stomach. In other instances, the animal was sacrificed and the stomach removed whole, the mucosa thoroughly rinsed with a dilute sodium bicarbonate solution, and the whole stomach wet-ashed. In a few experiments, the pylorus was clamped before the I^{131} injection. The blood concentration in counts per minute was read off the automatically recorded graph. After an initial drop in I^{131} concentration in the blood lasting about 6 minutes, the blood curve can be adequately described by the sum of two exponentials. The theory previously developed for two simultaneous rates of disposal (Dominguez, R., Goldblatt, H., and Pomerene, E. *Am. J. Physiol.*, 1937, **119**, 429–438) can be extended to the present study with three simultaneous rates under the assumption of proportionality of each rate to the instantaneous blood concentration. The renal clearances varied from hour to hour in every dog. The hourly variation in all 12 experiments was from 0.6 to 22.7 cc. per minute. The mean values per experiment varied from 1.9 to 19 cc. per minute with an over-all mean of 9.7. The average thyroid gland clearance was 1.16 cc. per minute. The average stomach clearance (gastric juice and gastric mucosa) was 7.1 cc. per minute. In some experiments the stomach clearance was larger than the renal clearance of I^{131}. The volume of body fluids penetrated by I^{131} during the first few minutes after injection was 3.06 liters (in terms of whole blood). The second volume penetrated in the first hour of the injection was 2.38 liters (also in terms of whole blood). The total volume of distribution of I^{131} was therefore 5.45 liters as whole blood. The average weight of the dogs of this study was 12.9 kg.

In three experiments with the pylorus clamped the amounts found in the stomach were 12.8, 17.3, and 21.5 per cent of the dose, respectively. Evidence has been obtained that, under the conditions of the experiments and without clamping the pylorus, very little I^{131} leaves the stomach during the first 3 to 4 hours after injection. Since, in the normal animal, the stomach eventually empties its secretion into the intestine and the intestine absorbs I^{131} readily, it follows that secretion into the stomach lumen acts as a temporary reservoir of iodine, the net effect of which is to

slow down its elimination. In future studies of iodine uptake by the thyroid gland, the iodine cycle of the stomach should be taken into consideration.

EFFECT OF VARIOUS SUPPLEMENTS ON INCIDENCE OF DIETARY CALCIFICATION IN C_3H MICE. Benjamin Highman and Floyd S. Daft (by invitation), National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, P.H.S., F. S. A., Bethesda, Md.

Abstract. As reported to this Association in 1951, weanling C_3H mice given diet 1926, a purified diet low in choline and vitamin E and containing 4 per cent of casein as the sole source of protein, died, frequently within 8 weeks. In addition to the anticipated fatty livers and cirrhosis, many of these animals developed calcified lesions in the arteries, particularly of the kidney, and in the heart, skeletal muscles, and lungs. The diet in subsequent experiments was modified to contain adequate choline, citrovorum factor, and vitamin B_{12} (diet 8137) without, however, preventing the development of these calcified lesions.

When the casein content of diet 8137 was increased to 10 or 25 per cent, the incidence of calcification was greatly diminished. These changes in incidence were statistically significant. For animals surviving 30 days or longer on the experimental diet, the following percentages had lesions as noted:

Per cent of casein	*Number of animals*	*Per cent with calcification*			
		Heart	*Muscles*	*Lungs*	*Arteries*
4	12	100	92	58	92
10	17	53	18	0	18
25	19	58	11	5	5

Since the muscle lesions resembled those of vitamin E deficiency and other lesions resembled somewhat those described for hypervitaminosis D, tests were made of the effect of increasing the vitamin E level and omitting the cod-liver oil (which increases the dietary need for vitamin E and also furnishes vitamin D). Neither of these changes separately had any statistically significant effects on the incidence of any of the lesions. When the cod-liver oil was omitted and there was a simultaneous supplementation of diet 1926 with alpha-tocopherol, the incidence of muscle lesions decreased. Decreases in incidence of lesions in the heart, lungs, and arteries were equivocal. It appears possible, therefore, that the muscle lesions bear a relationship to vitamin E deficiency. It is noteworthy that the incidence of muscle lesions was decreased to a greater degree by increasing the dietary casein.

Despite the significant decrease in incidence of all lesions when the level of protein was increased, it is to be noted that widespread calcification was still observed in some animals receiving as high as 25 per cent of casein. This suggests a possible deficiency in one or more unidentified factors. It is of some interest that the lesions in all organs (including vertebral muscles even after decalcification) stained intensely with the periodic acid-Schiff's procedure, resembling in this respect the cardiac and muscle lesions of potassium and other deficiencies.

STUDIES ON THE NATURE AND DEPOSITION OF KERNICTERIC PIGMENT. F. Stephen Vogel, the New York Hospital—Cornell Medical Center, New York, N.Y.

Abstract. Kernicteric pigment has been extracted by means of chloroform from the brains of 3 infants. Solutions of it proved strongly diazo-positive and, as determined electro-photometrically, gave maximum absorption of light having a wave length of 425 millimicrons, being identical in these respects with chloroform solutions of crystalline mesobilirubin. Solutions containing 1 to 1.5 mg. of crystalline mesobilirubin in 0.1 cc. of normal rabbit serum were injected intracerebrally into 10 newborn kittens, the animals being sacrificed at intervals up to 5 days. This stained the cerebral tissues at the sites of injection a bright canary yellow and

microscopic examinations showed that the pigment was deposited in great concentration in the nerve cells, although these were otherwise intact.

The fact has interest, in relation to the observations given above, that brain tissue can reduce bilirubin to mesobilirubin *in vitro*, as T. Baumgärtel has shown (*Klin. Wchnschr.*, 1946, **24–25**, 184–185). However, the question remains open why bilirubin crosses the blood-brain barrier in some but not in all infants with hyperbilirubinemia. Detailed clinical and pathologic studies have been made of 15 cases of kernicterus; immaturity or prematurity was present in 12, while impaired respirations with apnea had been observed in 13. Injections of 3.9 mg. of bilirubin in 1 cc. of normal rabbit serum were made slowly into the umbilical veins of living fetal rats at various stages of embryonic development, the flow of placental blood to the fetus being diminished for periods of 30 to 40 minutes. This was followed immediately by golden yellow pigmentation of the brains of the less mature fetuses, while, by contrast, the brains of the more mature fetuses were devoid of visible pigment.

The findings just given suggest that prematurity and anoxia may alter the blood-brain barrier so that bilirubin, when present in excess in the blood, may pass into the cerebral tissues, while the observations of Baumgärtel indicate that brain tissue can reduce bilirubin to mesobilirubin. It seems plain from the findings as a whole that kernicteric pigment is comprised of mesobilirubin. Yet it is also clear that neurons may be heavily pigmented, both in kernicterus and following the intracerebral injection of mesobilirubin, without exhibiting other cytologic changes.

AMYLOIDOSIS AND INCREASED BETA GLOBULINS OF SERUM IN RABBITS GIVEN RIBONUCLEOTIDE. Goetz W. Richter (by invitation), the New York Hospital—Cornell Medical Center, New York, N.Y.

Abstract. A relationship between abnormal serum globulins and amyloidosis has often been postulated, but no specific abnormality that is clearly related to the disease in man or animals has thus far been noted. Experiments already reported by the author have shown that the production of serum globulins in rabbits may be influenced by repeated injections of ribonucleotide (*J. Exper. Med.*, 1952, **96**, 331–346), while other workers have previously used such substances to produce amyloidosis in mice (Letterer, E. *Beitr. z. path. Anat. u. z. allg. Path.*, 1926, **75**, 486–588). To learn whether an amyloidosis that is regularly associated with specific alterations in the serum globulins might be produced in this way, rabbits were given subcutaneous injections of sterile 5 per cent sodium ribonucleate 5 times a week during 4 to 5 months, 10 cc. being given at each injection. After 4 to 5 months, all of 9 animals so treated developed amyloidosis of the spleen, as determined by detailed post-mortem examinations, while most of them also had amyloid deposits in the kidneys, and several had these elsewhere as well. The sera of these rabbits were repeatedly examined by means of electrophoresis on filter paper, and in several instances by the Tiselius method also. Total serum protein concentrations were determined by a micro-Kjeldahl procedure. After 1 to 3½ months of treatment, all 9 rabbits manifested two to threefold increases in the beta globulin levels of their blood sera, and these persisted until the animals were killed after 4 to 5 months of treatment, the elevations having then been present for a month or more in most instances. Most of the animals also had two to threefold increases in the levels of the alpha-2 serum globulins, but these were not always sustained. Except for occasional slight and transient rises during the first month of the experiments, the gamma globulins of these 9 animals remained normal.

As part of the same experiments rabbits were given frequent subcutaneous and intravenous injections of a foreign protein (bovine globulin) in order to produce sustained elevations of their gamma globulins and to determine whether the induced hyperglobulinemia would be associated with the development of amyloidosis. In all rabbits thus immunized the gamma globulins rose markedly and remained at levels

two to three times normal for periods of 3 to 4 months, but none developed amyloidosis. In other rabbits, pronounced simultaneous elevations of alpha-2, beta, and gamma globulins were produced by treatment with both ribonucleate and bovine gamma globulin during periods of 4 to 5 months. The animals treated in this way developed amyloidosis, but in most of them the disease was less advanced than that resulting in rabbits treated with ribonucleate alone. In further experiments the development of amyloidosis following subcutaneous injections of turpentine also was found to be associated with considerable rises of beta globulin levels.

The findings make it plain that amyloidosis can be produced regularly in rabbits by means of sodium ribonucleate. They also indicate that the formation of increased or abnormal beta globulins, perhaps together with increased or abnormal alpha-2 globulins, may lead in some way to the deposition of amyloid in tissues. The findings show further that the gamma globulins were not implicated in the pathogenesis of the amyloidosis here studied.

QUANTITATIVE CYTOPATHOLOGY: INTERFERENCE MICROSCOPY. Robert C. Mellors, Memorial Center, New York, N.Y.

Abstract. If it were possible to measure the thickness of a microscopic object as well as its area, it would be possible to compute the volume and the mass of the object. It was shown that the method of interference microscopy provides the depth and the lateral dimensions and that the thickness, the volume, the hydrous mass and the anhydrous organic mass of protoplasm in living, or unfixed, cells can be measured in absolute units such as μ, μ^3 and $\mu\mu$g. (10^{-12} gm.). The foregoing data can be provided for the nucleus and the cytoplasm of many types of cells, and, in favorable circumstances, the organic mass of small formed elements such as chromosomes and nucleoli can be estimated. The analytic potentialities of interference microscopy, together with the simplicity both of the method and of the equipment for its employment, are such that this should become a useful method in quantitative cytology and cytopathology. Cells in tissue culture, exfoliated cells, smears of bone marrow and peripheral blood, and cellular components separated from tissue masses provide suitable material for analysis. Representative studies of such material were presented.

INTRARENAL COMPRESSION. Howard J. Barrie, Banting Institute, University of Toronto, Toronto, Canada.

Abstract. Intratubular flow of urine is dependent on the correct intrarenal pressure, and for this reason the kidney is enclosed. The containment of the outer surface is effected simply by the capsule, and changes in renal volume during diuresis are achieved by changing the shape of the kidney more than by stretching the capsule. The containment of renal tissue in the concavity of the renal sinus is a more difficult problem. The columns of Bertin are invested by a strong fascia but this is the weakest point in the periphery and it is here that the kidney bulges when intrarenal tension becomes pathologic. The papillae are supported by the smooth muscle of the calyx. This muscle has a different structure to that usually ascribed to it. Its mode of action can be deduced from simple observation of necropsy material. It is closely related to the renal veins and it probably plays a key rôle in intrarenal compression.

Pathologic increases of intrarenal pressure may produce the following organic changes: 1, button herniation of the columns of Bertin; 2, herniation into renal veins; (a) hernia tubuli; (b) hernia spongiae; (c) hernia lymphaticae; 3, herniation of papillae with strangulation; 4, splitting of the capsule. Some of these changes lead directly to further increases in intrarenal pressure and a vicious circle may be established.

THE LYMPHATIC VASCULATURE OF THE HEART. Wilbur C. Thomas, Children's Hospital, Los Angeles, Calif.

Abstract. Relatively little attention has been paid to the rôle of the lymphatic system in diseases affecting the heart. Part of this neglect has been associated with an apparent general disregard for this system in consideration of diseases involving organs or the body as a whole, and much has been due to technical difficulties in studying the distribution of the lymphatic vessels. Simply demonstrated relationships have not received the attention they deserve. Color photographs of anatomical dissections and "revived heart" preparations illustrate the rôle of the "third circulation" of a vital organ in the pathogenesis of some of its diseases.

STUDIES ON THE RÔLE OF THE UNPROTECTED SPLEEN IN TOTAL BODY IRRADIATION. H. J. Van Baaren (by invitation) and D. Murray Angevine, School of Medicine, University of Wisconsin, Madison, Wis.

Abstract. Studies on the long range effect of relatively small doses of x-ray led us to the following observation: Two dogs receiving a weekly dose of 100 r. died during the experiment and showed severe pancytopenia in the peripheral blood combined with hyperplasia of the bone marrow. This observation, reminiscent of the findings in cases of human hypersplenism, directed our attention to the influence of the spleen on the blood picture in dogs subjected to total body irradiation. Studies in mice in which the spleen was shielded (Jacobson) showed a protective influence of the spleen, but in these experiments the protected spleen provided the animal with an intact source of lymphoid and reticulo-endothelial elements as well as a potential source of myeloid elements.

Four dogs were splenectomized and after the blood picture had returned approximately to the preoperative levels, they were subjected to 100 r. weekly until death. A control group of 4 animals was used for comparison. Weekly blood counts were done on both groups. The experiment has been repeated on a similar number of animals. No important differences in the total white counts of either group were observed until the last few weeks before death when the non-splenectomized dogs showed a more rapid decline in the total white count than did the splenectomized animals. The total neutrophil count paralleled the total white count, whereas the more rapid decline of the white count in the non-splenectomized dogs was mainly due to the drop in the band neutrophils. No differences in the lymphocyte counts in either group were observed. There were no appreciable differences in the red blood counts of either group until the last 2 to 4 weeks, when the decline in red count was more rapid in the non-splenectomized animals. No significant difference in time of survival was noted between the two groups.

EXCESSIVE "LIPOCHROME" PIGMENT IN LIVER CELLS IN "CONSTITUTIONAL HYPERBILIRUBINEMIA." Frank B. Johnson, Capt., M.C. (by invitation), and I. N. Dubin, Maj., M.C., Armed Forces Institute of Pathology, Washington, D.C.

Abstract. This report is based upon 9 cases of a disease entity characterized by the presence of excessive amounts of "lipochrome" pigment in liver cells in patients with the clinical picture of "constitutional hyperbilirubinemia." In the present series the clinical syndrome is that of hyperbilirubinemia in patients without hemolytic jaundice, biliary obstruction, or inflammatory disease of the liver. Absence of extrahepatic biliary obstruction was demonstrated at laparotomy in 4 cases in which this procedure was performed. On histologic examination, the liver was normal in all cases except for a striking amount of "lipochrome" pigment in parenchymal cells. This intracytoplasmic pigment was brownish yellow and granular. It was not extractable by either polar or non-polar solvents. Tests for iron by the Gomori method and by microincineration were negative, as were tests for bile by the Stein and Gmelin reactions. It contains 1,2 glycols or glycol substituents as demonstrated by the periodic acid-

Schiff's method and it contains ethylenic linkages as shown by the performic Schiff reaction. It differs from ceroid in that it lacks (1) significant acid-fastness, (2) striking fluorescence under ultraviolet microscopy, and (3) sudanophilia. This pigment more closely resembles the group of "lipochrome" pigments than any other category.

Consideration is given to the possibility that this hepatic abnormality is the histologic counterpart of the clinical entity which has been variously described as "constitutional hyperbilirubinemia," "constitutional hepatic dysfunction," "icterus intermittens juvenilis," and "familial non-hemolytic jaundice." In previously reported studies in which liver biopsies were made, however, the liver was said to be histologically normal, although in one case the pathologist reported the presence of an unusual amount of pigment in liver cells which he considered to be neither iron nor bile pigment. Proof that the lesion described in our series of cases represents the characteristic histopathologic picture of "constitutional hyperbilirubinemia" awaits further studies now being pursued.

THE EFFECT OF VARIATIONS IN BASIC DIETARY COMPONENTS ON THE FATE OF LOCALIZED FIBROSIS IN THE LIVER, INDUCED BY THE IMPLANTATION OF PLAIN SURGICAL GUT. Henry Ungar (by invitation), Hebrew University—Hadassah Medical School, Jerusalem, Israel. (Temporary address: Department of Pathology, University of California School of Medicine, San Francisco, Calif.)

Abstract. A method of studying the reaction of fibrous tissue and the disposition of collagen implanted in the liver has been reported previously. By this method a thread of homologous rat tendon or plain surgical gut (sheep collagen) was fixed in a lobe of the liver. Homologous tendon remained intact but stimulated the development of a narrow capsule of vascularized, newly formed connective tissue for at least 2 months. Surgical gut, however, was absorbed within 3 weeks and stimulated the proliferation of collagenous connective tissue, which regressed leaving a minute scar. With variations in the diet, the tissue reaction following implantation of surgical gut was modified as follows: 1. The implanted gut was absorbed within about 3 weeks in the rats on a low protein-22 per cent fat diet; and, during the second month of the experiment, on a high carbohydrate-low fat diet. In rats on a low protein-40 per cent fat diet the gut remained unabsorbed during an observation period of 2 months. 2. Regression of newly formed fibrous tissue within 2 months was observed in animals on a low protein-22 per cent fat diet and on a high carbohydrate-low fat diet. No regression occurred in animals on a low protein-40 per cent fat diet. 3. Addition of lipotropic substances to the low protein-22 per cent fat diet does not prevent the formation or accelerate the absorption of fibrous tissue.

The technique of implantation offers several advantages in the study of regression of hepatic cirrhosis, which has been suggested by the observation of several authors: First, the quantity of collagen presented to the liver for absorption can be controlled. Second, there is a single quantitative stimulus for the formation of reactive fibrous tissue by the liver. Third, the temporal sequence of events can be accurately ascertained.

THE PITUITARY GLAND IN PATIENTS TREATED WITH CORTISONE AND ACTH. Warren A. Bennett and R. A. Kilby (by invitation), Mayo Clinic, Rochester, Minn.

Abstract. This is a preliminary report on material from 77 patients treated with cortisone or ACTH or both. It was noted in pituitary bodies stained by Crooke's method that the administration of cortisone had caused certain changes similar to those described by Crooke. The changes in question are considered by most pathologists to constitute a degenerative process seen in Cushing's disease, termed "pituitary basophilism," and also seen occasionally in basophilic adenomas of the pituitary body, and in patients with adrenal virilism, and virilizing tumors of the ovary. In our

cases it was noticed that the initial change in the basophils of the pituitary body was clumping of the granules. In consonance with this change, the cytoplasm became reticulated and finally assumed a ground-glass appearance. During these processes vacuoles occurred, and the terminal event was hyalinization with vacuolization.

The important features are the duration of treatment and the dose administered. A total dose of 400 mg. in a 4-day period is adequate to cause atrophy of the adrenal gland and significant changes in the pituitary body. One patient who had not received cortisone for 15½ months was found to have moderate atrophy of the adrenal glands and maximal changes in the basophils of the pituitary body. These changes were accompanied by atrophy of the entire pituitary body. Among patients treated with ACTH the same alterations in the basophils of the pituitary body occurred. There appeared to be an earlier hyalinization and vacuolization than in those treated with cortisone. In 11 of the 77 patients evidence of hypercorticalism was seen, and in all these the adrenal glands were atrophic and the pituitary body showed the basophilic alterations to complete hyalinization. It is therefore apparent that some change occurs in the pituitary body and in the adrenal glands as a result of the administration of cortisone and ACTH. The use of cortisone results in atrophy of the adrenal glands, with degenerative changes in the basophils of the pituitary body, while with ACTH there are more severe degenerative changes in the pituitary body and hyperplasia of the adrenal cortex.

FRACTIONATION OF MAMMALIAN PITUITARY CELLS BY DIFFERENTIAL CENTRIFUGATION: ISOLATION AND BIOLOGIC ACTIVITY OF CYTOPLASMIC GRANULES. Donald D. Mark (by invitation), Johns Hopkins Hospital, Baltimore, Md.

Abstract. The biologic effects known to be regulated by the adenohypophysis have long been related to the number and kind of cells present, which is determined by the number and kind of particulate cytoplasmic components (mainly stainable granules). Although the granules are thought to represent the secretory product of the adenohypophysis, direct proof of hormonal activity is lacking. The present report deals chiefly with the segregation of a particulate cytoplasmic component derived from anterior pituitary gland homogenates. It has been possible by means of differential centrifugation to separate a morphologically distinct "granule" fraction in large yield. Only this fraction was considered because light and electron microscopic studies show that it may be related to the formed elements as they exist in the cytoplasm. Bio-assays which were performed on immature hypophysectomized female rats, using the granules from beef adenohypophysis and known pure beef anterior pituitary hormones as control preparations, show that gonadotropic, thyrotropic, adrenotropic, and growth-stimulating activity exists in these granules. No centrifugal or other separation has been possible as yet between acidophilic and basophilic granules. It is noteworthy, however, that counts of steer pituitary gland (from which the experimental material was derived) show that the ratio of acidophils to basophils is 28:1.

CYTOCHEMICAL STUDIES ON BACTERIA AND FUNGI. Walter Joel (by invitation), University of Oklahoma School of Medicine, Oklahoma City, Okla.

Abstract. Cytochemical methods for demonstrating neutral and acid polysaccharides have been applied to various bacteria and fungi, including Streptococci, Pneumococci, *Candida albicans, Saccharomyces cerevisiae, Coccidioides immitis, Cryptococcus hominis,* Blastomyces, *Histoplasma capsulatum,* and *Sporotrichum schencki.* Several of these fungi exhibited strikingly different behavior to stains for acid and neutral polysaccharide. In coccidioidomycosis, blastomycosis, and sporotrichosis the organisms are best demonstrated by procedures which stain neutral polysaccharides. The organism of histoplasmosis stains by the Hale-Rinehart procedure for acid polysaccharide. This same procedure also stains deeply the capsule of

Cryptococcus hominis. *Candida albicans* and *Saccharomyces cerevisiae* present interesting features after application of the combined stain for acid and neutral polysaccharides. *Candida albicans* exhibits a blue halo (capsule?) around the red organism. Saccharomyces is stained only red, hence this cytochemical procedure readily differentiates organisms which are morphologically almost identical. Cytochemical methods for demonstrating fat also were applied to bacteria and fungi. Benzpyrene-stained sections studied by fluorescent microscopy disclosed bound lipids and this method was found to be especially valuable in studying tubercle bacilli.

OBSERVATIONS ON SENILE CEREBRAL DEPOSITS USING THE PERIODIC ACID-SCHIFF'S TECHNIQUE. George Margolis, Duke Medical School, Durham, N.C.

Abstract. The periodic acid-Schiff's (PAS) technique was employed in a study of argentophilic deposits in senile brains of man. Correlation of these observations with the results obtained by the classical metallic techniques resulted in a clear definition of four distinct elements composing senile plaques. These components were: amorphous, fibrillary, cellular, and reticular. These elements were given a monochromatic rendition by the classical methods. The PAS technique consistently demonstrated the amorphous component in the plaques, and separated it from the other elements. The cellular elements were PAS positive only by virtue of their granular inclusions. The reticular and fibrillar elements were PAS negative, but were sometimes demonstrated in part by a deep, well differentiated hematoxylin counterstain. The vascular network of the brain was brilliantly outlined by the PAS positive basement membrane. Perivascular deposits gave reactions identical to the amorphous component of senile plaques, both in the PAS and the metallic techniques. There was no further resemblance between the senile plaques and the perivascular depositions.

These findings support the concept that senile plaques are the result of a deposition of colloids in the ground substance of the cerebral cortex. They also contribute evidence supporting the concept that these deposits occur about disintegrating neurons which have been altered by the neurofibrillary change of Alzheimer. The cellular components are interpreted as reacting glial cells. The reticular elements, not demonstrable in all plaques, are interpreted as secondary deposits of basophilic materials upon the amorphous component.

THE PATHOLOGIC SIGNIFICANCE OF SPARSELY GRANULATED PAS POSITIVE CELLS IN THE HUMAN ANTERIOR PITUITARY GLAND. Agnes S. Burt (by invitation), Massachusetts General Hospital, Boston, Mass.

Abstract. Application of the periodic acid-Schiff's technique to the human anterior pituitary gland shows that, in addition to the PAS positive normal basophil, there is a second type of sparsely granulated PAS positive cell which is present in small numbers in endocrinologically normal individuals and is significantly increased in a number of altered physiologic states. This is illustrated by findings in adrenal hyperplasia (10 cases), pregnancy (6 cases), ovarian stromal hyperplasia (6 cases), interstitial cell hyperplasia (1 case), thyroidectomy, propyl thiouracil or I^{131} treatment (7 cases), and thyroid aplasia (1 case). The data suggest that cells of this type may be concerned in the production of some adrenocorticotrophic factor, a gonadotropin (possibly LH) and thyrotropic hormone. The discrepancies in pituitary staining with PAS and Mallory techniques were discussed and offered as a possible explanation of some contradictory findings in the older literature.

CORONARY ARTERY LESIONS IN SUDDEN DEATH. Stanley H. Durlacher, Arthur J. Fisk (by invitation), Louisiana State University School of Medicine, New Orleans, La., and Russell S. Fisher, University of Maryland School of Medicine, Baltimore, Md.

Abstract. The coronary arteries of adults who die suddenly have been examined

by a technique of perfusion and fixation before the major ramifications of the vessels are dissected from the heart. Decalcification, dehydration, and clearing of the vessels are accomplished before examination with the dissecting microscope. Cross sections are made and selected areas are embedded and studied microscopically. The experimental group included only adults under the age of 60 who died suddenly and unexpectedly, with no gross extracardiac cause of death revealed by complete necropsy. From this group cases were eliminated that had demonstrable lesions responsible for death after microscopic examination of all tissues other than the coronary arteries. A control group comprised persons of similar age, sex, and race whose sudden death was found to be due to causes other than coronary artery disease. Hemorrhages within atheromatous or fibrous plaques of the coronary arteries were found in all cases of the experimental group. The hemorrhages were multiple in almost all instances, and varied in age from fresh extravasations to areas of iron-containing pigmentation. All branches of the coronary arborization were involved and the hemorrhages were usually non-occlusive. Thrombi were frequently associated with the older hemorrhages but not with the fresh ones. Fresh mural hemorrhages were not observed in the control series and only an occasional instance of pigment deposition was found in this group.

AGING (WEAR AND TEAR) PATTERNS IN ARTERIES AS RELATED TO PHYSICAL FACTORS AND ANATOMICAL LOCATION. Herman T. Blumenthal, Jewish Hospital and St. Louis University School of Medicine, St. Louis, Mo.

Abstract. In a previous series of reports the effects of aging (wear and tear) on the elastic structures of arteries were described. The present report deals with a comparative study of the severity of these changes in various major arteries at corresponding age periods. The arteries studied include the coronaries, hepatic, splenic, renal, iliacs, basilar, pulmonary, and various segments of the aorta and of the arterial tree of the lower extremities. In general, the severity of elastic tissue alterations can be related primarily to the internal hydrostatic pressure which is a function of the internal diameter of the artery and the blood pressure, as well as to gravitational effects due to the upright human position. The relationship of these effects to the formation of intimal atherosclerotic plaques and vascular thrombosis was discussed.

A STUDY OF THE HUMAN AORTA DEALING WITH THE RÔLE OF MUCOPOLYSACCHARIDES IN THE PATHOGENESIS OF INTIMAL FIBROSIS AND ATHEROSCLEROSIS.* H. E. Taylor, University of British Columbia Medical School and the Vancouver General Hospital, Vancouver, Canada.

Abstract. Recently, attention has again been focused on the rôle of mucopolysaccharides in the pathogenesis of atherosclerosis, by reports on pyridoxine-deficiency arteriosclerosis in monkeys and on human coronary sclerosis (Rinehart, J. F., and Greenberg, L. D. *A. M. A. Arch. Path.*, 1951, **51**, 12–18; Moon, H. D., and Rinehart, J. F. *Circulation*, 1952, **6**, 481–488). The present report deals with the human aorta. Tissue was studied in all age groups from areas grossly free of atherosclerotic plaques, particular attention being paid to the relation of the intercellular substance and elastic tissue. The former stained metachromatically with toluidine blue, was specifically inhibited by hyaluronidase, was unaffected by ribonuclease, and resisted fixation in boiling methanol-chloroform. Periodic acid-Schiff's reagent did not react with the masses of metachromatic substance, but did reveal a further delicate fibrillar substance between the elastic fibers. In this abstract, the metachromatic substance will be referred to as mucopolysaccharide, to which group it appears to be closely allied.

In the first decade of life this mucopolysaccharide was diffusely distributed through the media, in later life it became more concentrated in focal areas, appearing as

* This article will appear in a subsequent issue of *The American Journal of Pathology*.

clumps scattered between the elastic fibers. Whenever elastic fibers were fractured, it was seen as bubbly masses between the ends of the fibers and when fragmentation of elastica was prominent, *e.g.*, with medionecrosis, a pool of mucopolysaccharide accumulated in the area. In all areas of intimal fibrosis a varying degree of elastic fragmentation occurred in the immediately adjacent media and here a collection of mucopolysaccharide formed a distinct metachromatic zone. Lipid was deposited in variable amounts within the intima, but it was present also in the areas of medial degeneration, sometimes closely applied to the elastic fibers, at others within scattered lipophages. In the more advanced phases it was apparent that "intimal" hyaline thickening was due in part to this medial degeneration. The hyalin frequently showed an admixture of mucopolysaccharide and lipid; the former apparently derived from the blood serum. It is postulated that this profound alteration in mucopolysaccharide content and distribution may be important in the further development of atherosclerosis, either by altering the permeability of the vessel wall or through an affinity of the mucopolysaccharide for other chemical constituents such as lipoprotein.

HISTOCHEMICAL STUDIES OF ACUTE VASCULAR LESIONS IN BILATERALLY NEPHRECTOMIZED DOGS. P. O'B. Montgomery and E. E. Muirhead, Southwestern Medical School of the University of Texas, Dallas, Texas.

Abstract. Histochemical studies of the acute vascular lesions occurring in bilaterally nephrectomized dogs were presented to demonstrate that these vascular lesions are similar to those occurring in humans with malignant hypertension and that these lesions are due in large part to necrosis of the smooth muscle of the vessel wall. The standard stains for collagen, muscle, elastic tissue, reticulum, and fibrous tissue were presented in an introductory fashion. Following these, lesions histochemically treated to demonstrate fats, lipoprotein, phospholipids, cholesterol, free carbonyl groups, esterase, acid and alkaline phosphatase, mucin, mucopolysaccharides, protein bound sulfhydryl groups, amyloid, and acid-fast material were presented. Results from these stains may be interpreted as showing that these acute vascular lesions are the consequence of necrosis of the smooth muscle of the vessel wall, and that the so-called hyalin of these lesions is, in reality, made up of necrotic smooth muscle in varying stages of fusion.

OBSERVATIONS IN TISSUE CULTURE OF HUMAN TISSUES NATURALLY INFECTED BY HISTOPLASMA CAPSULATUM. Charles C. Randall and Alice L. Hackney (by invitation), Vanderbilt University School of Medicine, Nashville, Tenn.

Abstract. It has been previously documented by one of the authors (C.C.R.) that chick and horse tissues maintained *in vitro* could be infected experimentally with the yeast-cell phase of *Histoplasma capsulatum*. Numerous proliferating fibroblast-like cells were observed to contain yeast cells. In the present study it was deemed of interest to study known and suspected cases when available. Spleen from a necropsied case of disseminated histoplasmosis and tissue obtained from an infant whose spleen was removed for prophylactic reasons were studied by the stationary-slide and suspended-cell methods of tissue culture. Repeated mycologic cultures were negative before splenectomy. Tissues maintained by the several tissue culture techniques from both cases were stained at intervals. Marked increase in intracellular development of yeast cells was noted. The two tissue culture methods and routine cultures, and routinely stained and sectioned tissues were compared. In doubtful cases tissue culture methods may be valuable diagnostic tools.

INTRACELLULAR PROTEIN INCLUSIONS IN LYMPHOMAS ASSOCIATED WITH ACQUIRED HEMOLYTIC ANEMIA. Henry Rappaport, Armed Forces Institute of Pathology and Veterans Administration Central Laboratory, Washington, D.C.

Abstract. In a study of the pathologic features of acquired hemolytic anemia,

abundant intracytoplasmic acidophilic bodies were observed in 3 cases in which the disease was associated with either chronic lymphocytic leukemia or lymphosarcoma. These acidophilic bodies had the staining qualities of protein, gave a strongly positive reaction with the periodic acid-Schiff's method, and resembled Russell bodies tinctorially, histochemically, and, to varying degrees, morphologically. They were found in neoplastic lymphocytes in lymph nodes, spleen, bone marrow, and visceral leukemic infiltrations and, in one instance, also in reticulo-endothelial cells filling the lymph node sinuses. Plasma cells were not recognized in the lymph nodes or in the leukemic infiltrates. In all 3 patients the Coombs test was positive on repeated occasions, and in 2 warm auto-agglutinins in very high titers were demonstrated. In view of these observations the following interrelated possibilities were considered: (1) That these protein inclusions may be produced by the cells containing them, which would be comparable to the formation of Russell bodies by plasma cells and multiple myeloma cells. (2) That in some instances of malignant lymphoma associated with acquired hemolytic anemia the neoplastic lymphocytes may be the site of production of abnormal proteins similar to the formation of Bence Jones protein by multiple myeloma cells. (3) That these abnormal proteins may in some way be related to the auto-antibodies occurring in acquired hemolytic anemia. While these hypotheses could not be proved conclusively on the basis of the available material, the observations reported here suggest that further studies directed toward more precise identification of these intracellular proteins may shed light on the site of production of auto-antibodies in acquired hemolytic anemia associated with malignant lymphoma.

A Routine Method for the Histopathologic Study of the Human Sino-atrial Node.* Maurice Lev and A. Watne (by invitation), the Mount Sinai Hospital of Greater Miami, Miami Beach, Fla., and the University of Illinois, College of Medicine, Chicago, Ill.

Abstract. There is at present no routine method for the histopathologic study of the sino-atrial node. The present work is an attempt at devising such a method. Previously, the senior author had devised a method for the study of the atrioventricular node, bundle, and bundle branches (Lev, M., Widran, J., and Erickson, E. E. *A. M. A. Arch. Path.*, 1951, **52**, 73–83; Widran, J., and Lev, M. *Circulation*, 1951, **4**, 863–867; Erickson, E. E., and Lev, M. *J. Geront.*, 1952, **7**, 1–12).

The method of study was as follows: (1) The superior cavo-atrial region was studied grossly in various age groups. Sixty-seven formalin-fixed hearts were dissected originally. (2) Histologic study was conducted on 53 other formalin-fixed hearts of varying ages, with hematoxylin-eosin stain, Weigert's elastic tissue counterstained with van Gieson's connective tissue stain, Hortega's reticulum stain, and Mallory's phosphotungstic acid hematoxylin and Gomori's trichrome stains for myofibrils, cross striations, and intercalated disks. (3) Sixty-three hearts were now dissected and studied more minutely grossly. By the above procedures the exact position and environment of the node were ascertained. (4) A method of sectioning was now devised giving 100 per cent continuity of structure in 50 sections.

Although the sino-atrial node region could be clearly dissected, the boundaries of the node could not be clearly demarcated grossly. On the other hand, histologically the node is a distinct structure and easily identified. Its differentiating characteristics are: (1) The fibers are relatively small and in plexiform arrangement. (2) The myofibrils are relatively scant with few cross striations. (3) There is a very large amount of collagen surrounding the fibers, and a very prominent elastic network. The node grows more slowly than the atrial musculature. With aging it shows an increase in connective and elastic tissue and, in some cases, in fat tissue.

* This investigation was supported by a grant from the Heart Association of Greater Miami, and by a grant (H-430 C_2) from the National Heart Institute of the National Institutes of Health, United States Public Health Service.

PRE-INVASIVE CARCINOMA OF THE CERVIX: A SERIAL BLOCK SURVEY. Robert P. Carson (by invitation) and Edward A. Gall, University of Cincinnati College of Medicine, and Cincinnati General Hospital, Cincinnati, Ohio.

Abstract. Large tissue sections were made from 15 uteri which were resected following diagnosis by biopsy of pre-invasive squamous cell carcinoma. An average of 12 blocks were prepared in radial serial fashion from each specimen, thus permitting a panoramic view of the distribution and nature of the process. It was apparent that the lesion was uncommonly a limited one, for in each specimen it was found to be widely distributed. The several patterns encountered were:

(a) broad, plaque-like, non-invasive lesions	7
(b) multiple independent non-invasive focal lesions	7
(c) one or more foci of early invasion	2
(d) unsuspected advanced invasion	3

Though widely distributed within the endocervix there was no example of significant extension of the neoplasm on the vaginal surface of the cervix. Of particular interest was the frequent concomitance of the peculiar lesion termed by others "basal cell hyperplasia," "atypical metaplasia," or "precancerous cervicitis." This was found coexistent with frank neoplasm in 13 of the 15 cases in this series. Criteria were established to distinguish it from ordinary squamous metaplasia on the one hand and pre-invasive carcinoma on the other. It is our belief that this represents a reversible alteration which, when encountered, indicates need for continued investigation.

CARCINOMA IN SITU AND ITS RELATION TO INVASIVE CANCER. James W. Reagan and Dorothy J. Hicks (by invitation), Institute of Pathology of Western Reserve University and the University Hospitals of Cleveland, Cleveland, Ohio.

Abstract. This comprehensive study of carcinoma *in situ* deals with the (1) incidence, (2) prevalence, (3) age, (4) racial and (5) religious distributions, (6) morphology, (7) anatomical lesions, (8) histogenesis, and (9) biologic activity of the lesion. It was undertaken in order to make a more accurate comparison with the findings in frank cancer of the cervix and in atypical hyperplasia or dysplasia. The study was based on 5,044 complete cervices or portions thereof examined in this institution during a 5-year period. Included were 100 cases of carcinoma *in situ*, 225 cases of invasive cancer, and 133 lesions designated atypical hyperplasia.

ON THE ORIGIN AND DEVELOPMENT OF MIXED TUMORS OF SALIVARY GLANDS. Robert P. Morehead and Robert E. Klein (by invitation), Bowman Gray School of Medicine, Winston-Salem, N.C.

Abstract. With special reference to their origin and development, a large group of neoplasms originating in both major and minor salivary glands has been studied. Parallel studies have been made of other groups of tumors which were structurally identical with the salivary gland neoplasms but which arose in locations quite remote from these structures. Evidence has been obtained which strongly supports the contention that mixed tumors of salivary glands arise from salivary epithelium. This epithelium is morphologically indistinguishable from that which is normally present in these structures. As the tumors develop, it becomes evident that these cells are capable not only of proliferation but also of pluripotential development. In each instance, however, the cells which compose the parenchyma of the tumor are embryologically indigenous to the area in which they arise. The cells exhibit in an abnormal manner their latent ability to differentiate into all of those structures normally attributed to the ectoderm from which they originally arose. In many combinations and with varying degrees of completeness, this variable cellular differentiation results in tumors composed of structures resembling (a) basal cell tumor, (b) adenocystic

basal cell tumor, (c) mucin-producing cells and epithelial mucin, (d) pseudocartilage, (e) mucin-producing cells arranged in an alveolar pattern, (f) focal myoepithelial cell proliferation, (g) non-keratinized and keratinized stratified squamous epithelium, (h) sebaceous glands, and (i) hair follicles.

It would appear that under both physiologic and pathologic conditions, proliferating cells produce variable changes in adjacent tissues. It is not surprising, therefore, that one observes excessive proliferative activity and, at times, metaplasia of the stroma of these tumors. From the loose connective tissue cells of the stroma come fat, true cartilage, bone, lymphoid tissue and other heterotopic structures. In studying these tumors one must separate sharply the neoplastic components which comprise the parenchyma of the tumor from the metaplastic ones which are derived from the stroma.

The validity of these observations regarding mixed tumors of salivary glands was further strengthened by study of a large group of tumors of identical structure which arose in locations quite remote from both minor and major salivary glands. The most impressive of this group were those neoplasms which originated within the skin.

SYMPOSIUM ON THE MODIFICATION OF STRUCTURAL CHANGES IN INFECTIOUS, NEOPLASTIC, AND OTHER DISEASES FOLLOWING THE USE OF MODERN CHEMOTHERAPEUTIC AGENTS

EXPERIMENTAL BACTERIAL ENDOCARDITIS IN ALTITUDE RATS. PATHOLOGIC LESIONS AND EFFECT OF PENICILLIN THERAPY.

Benjamin Highman, Paul D. Altland (by invitation) and Harry Eagle (by invitation), National Institutes of Health, Bethesda, Md.

Abstract. Young male Holtzmann rats numbering 247 were exposed 4 hours daily to a simulated altitude of 25,000 feet and then inoculated intravenously with a young broth culture of *Streptococcus faecalis.* Numerical details and incidence of lesions have been reported in part elsewhere (*Proc. Soc. Exper. Biol. & Med.*, 1952, **81**, 135–139). In this report, pathologic details are emphasized.

Severe cardiac lesions were uncommon in rats treated with penicillin beginning 12 and 20 hours after inoculation. Untreated rats generally developed bacterial vegetations involving chiefly the mitral valve, less frequently the aortic, and occasionally the tricuspid or pulmonary valves or the mural endocardium. The valves of rats killed 12 hours after inoculation often showed a focal sub-endothelial infiltration by polymorphonuclear leukocytes, edema, and sometimes one or more small desquamated areas covered by fibrin. After 20 hours, the changes were usually more severe with a widespread leukocytic infiltration of the affected valves often extending into the root of the aorta, the valvular rings, and the adjoining myocardium. At 108 hours, when treatment was instituted using 200 mg./kg. sodium penicillin G four times daily for 2 to 11 days, nearly all rats studied had a severe valvulitis with extensive ulceration and large fibrinous vegetations containing masses of bacteria. There were often areas of suppuration in the valvular rings and at the base of the vegetations, but relatively few leukocytes in the immediate vicinity of the bacteria. The severity of the lesions and the rate of regression after beginning therapy varied considerably in different animals. Rats killed 24 to 48 hours after beginning therapy often showed some fibrous organization of the vegetations with relatively few or no demonstrable bacteria. Organization of the vegetations sometimes appeared nearly complete within 6 days after beginning therapy. Most rats killed at later intervals displayed only slight or no evidence of previous valvulitis, but a few showed persistent severe bacterial infection, persistent lesions in the valvular rings, or fibrous

thickening and distortion of the valves. Similar changes were noted in a few untreated survivors killed after the second week; those dying earlier had severe lesions.

The kidneys of untreated rats killed 36 to 60 hours after inoculation generally showed scattered inflammatory foci in which the tubules contained a purulent exudate occasionally mixed with bacteria. A few showed coagulation necrosis in the renal papillae with masses of bacteria and some leukocytes. Such lesions became more numerous and severe after 60 hours. Rats treated with penicillin beginning 12 and 20 hours after inoculation showed no renal lesions or only small inflammatory foci; only one rat, rested 34 days after treatment for 1 day, showed renal necrosis. When penicillin therapy was instituted 108 hours after inoculation, regression of renal lesions was much slower than in the heart. Bacterial colonies and necrosis persisted in the renal papillae in one rat killed immediately after receiving treatment for 8 days. Similar lesions were noted in one of 4 rats treated 4 days and killed 36 days after inoculation. Each of 11 other rats killed at this time showed regressing renal lesions evidenced by depressed scars infiltrated by lymphoid cells, with purulent exudate in a relatively few tubules. Similar lesions were noted in untreated rats surviving more than 2 weeks.

CHANGING MORPHOLOGIC PICTURE OF ENDOCARDITIS AT NECROPSY SINCE THE ADVENT OF CHEMOTHERAPY AND ANTIBIOTIC AGENTS. Alfred A. Angrist and Jeanne Marquiss (by invitation), Queens General Hospital, Jamaica, N.Y.

Abstract. The incidence of the different forms of endocarditis encountered at necropsy has changed remarkably since the use of chemotherapy and antibiotics. Data were presented to note the marked decrease in bacterial endocarditis and the corresponding increasing incidence of the non-bacterial forms of the disease, particularly thrombotic non-bacterial endocardioses. The significance of this altered incidence for the understanding of the pathogenic mechanism of the classical forms of endocarditis was stressed.

In a previous communication the significance of the frequently encountered transitional forms of endocarditis, intermediate between the classical textbook lesions, has been noted from the standpoint of pathogenesis. Whereas such transitional lesions were encountered only infrequently heretofore, in the modern therapeutic era the bacterial vegetations encountered at necropsy, more often than not, show graded transitional features between the bacterial and the non-bacterial forms, and between the acute and subacute forms, and also show gradations of the healing phases of such lesions. On the basis of the change in incidence and the study of such transitional lesions, particularly between the non-bacterial and the bacterial vegetations, it is suggested that the primary lesion in all endocarditis is the equivalent of a thrombotic non-bacterial vegetation. The pathogenesis of all bacterial forms of endocarditis is suggested as the result of a superimposed bacterial infection upon a primary initial thrombotic non-bacterial vegetation. This basic lesion is considered the result of an alteration of the valvular collagen, presented as a somewhat variable, fundamental, biologic, enzymatic, biochemical disorder of the ground substance of valvular collagen matrix. Some of the factors controlling such collagen reactivity, with its tendency to necrosis or variable depolymerization, include the nutritional state, endocrine function, and the reaction to stress. The experimental production of endocarditis by the use of non-specific stress was demonstrated.

EFFECT OF STREPTOMYCIN ON TUBERCULOUS MENINGITIS. Webb Haymaker and Hartwig Kuhlenbeck (by invitation), Armed Forces Institute of Pathology, Washington, D.C.

Abstract. Forty-five cases of tuberculous meningitis were studied post mortem. All had received streptomycin both intramuscularly and intrathecally. In 14, the survival period was 6 months or longer, with a maximum of 17 months. In the

spinal meninges at later stages only mononuclear cells persisted, indicating a lytic effect of the streptomycin on the exudate. Associated fibrosis was usually slight to moderate.

In the cerebral meninges at later stages many regions had been cleared of exudate, but in virtually all cases pockets of fibrino-caseous exudate had persisted and been walled off by an abundant growth of connective tissue. Such walled-off pockets of exudate, most common at the midbrain level, may have remained unresolved because of their location, coupled with a comparatively low concentration of streptomycin in the cerebrospinal fluid. Terminally, in most of the cases, acute meningitis, with the formation of tubercles, had occurred, especially in the region of the brain stem. In 4 cases, however, no evidence of terminal flare-up of the meningitis was noted. In one case there was virtually complete healing of the meninges unattended by appreciable fibrosis. The patient lived 7 months after the onset of the meningitis, only to die from massive pulmonary hemorrhage.

Hydrocephalus was prominent in most of the cases. Sites of obstruction were as follows: aqueduct in 5, foramina of Luschka in 4, and aqueduct and foramina of Luschka in 7. Communicating hydrocephalus was present in most of the other cases.

Another feature of interest was the massive necrosis (not infarction) of the striatum and/or pallidum in 6 cases. This was regarded as due to ischemia brought about by reduced blood flow through narrowed vessels.

PATHOLOGIC CHANGES IN TUBERCULOSIS FOLLOWING THE USE OF STREPTOMYCIN THERAPY. Oscar Auerbach, Veterans Administration Hospital, East Orange, N.J.

Abstract. The prolonged use of streptomycin alters greatly the disease process in tuberculosis. It exerts its greatest influence on superficial surfaces. The perifocal reaction around the tuberculous foci clears rapidly. Ulcers of the larynx, tracheobronchial tree and intestines heal quickly with re-epithelization. Ulcers of the bronchus at the bronchocavitary junction invariably heal under the influence of these agents. The newly formed epithelium (invariably squamous) extends onto the inner wall of the cavity for some distance. The contents of the cavity inspissate and in cases with prolonged chemotherapy tubercle bacilli, although stainable, cannot be grown on culture media or produce disease in animals. Neutrophils in large numbers may invade the inspissated necrotic contents by way of the patent bronchus and result in liquefaction of this material with subsequent evacuation, resulting in a cyst lined either by epithelium or by a layer of hyalinized connective tissue. In untreated cases cavity closure occurs secondary to an obliteration of the bronchus by union of the ulcerated surfaces at the bronchocavitary junction, generally with the aid of artificial pneumothorax or thoracoplasty.

The natural course of tuberculous meningitis is prolonged and benefited by streptomycin therapy. The exudate within the subarachnoid space undergoes extensive necrosis and vessel walls are partially involved in a similar process. Tuberculous granulation tissue which develops from the pia-arachnoid replaces the remaining fibrinocellular exudate in the subarachnoid space so that, in cases receiving prolonged therapy, the base of the brain is covered by a dense layer of hyalinized connective tissue which encloses necrotic foci. The favorable effects of the chemotherapeutic agents cease when the organisms become resistant to the drug.

FATAL FULMINATING STAPHYLOCOCCIC GASTRO-ENTEROCOLITIS WITH SHOCK-LIKE STATE FOLLOWING ANTIBIOTIC TREATMENT. Kornel Terplan, Buffalo General Hospital, Buffalo, N.Y.

Abstract. Within 2 months (May and June of 1952) 6 deaths occurred in several hospitals in Buffalo in patients in whom various abdominal surgical procedures were performed, accompanied by administration of the following antibiotics: terramycin in 2, penicillin and streptomycin (syncrobin) in 3, and penicillin, terramycin, and

aureomycin in one. The causative agent responsible for the acute desquamative and membranous enterocolitis terminating in a shock-like state was demonstrated first by histologic methods in the exudate within the lumen and in the floating membranes covering the surface of the acutely inflamed intestinal tract. In one instance, 10 days after the preservation of the intestine in deep freeze, massive pure growth of coagulase-positive hemolytic staphylococcus was obtained. Cultures for enteric pathogens had been consistently negative. Of 3 other cases, staphylococcus was the predominating organism in 2; in the other, it was combined with a few colonies of enterococci and proteus. In smears of the watery intestinal content, masses of gram-positive cocci were seen and no other microorganisms. In 2 additional cases of fatal enteritis and enterocolitis, which had developed following aureomycin medication, hemolytic staphylococcus was cultured from the small intestine in one. In both, masses of gram-positive cocci could be demonstrated within the membrane of the colon and in the exudate covering the mucosa of the small intestine. One of these cases had been observed 4 years, the other 1 year previously.

In all, the clinical course and pathologic pictures were similar. Nausea and vomiting preceded the rapidly progressing watery diarrhea, commencing from the first to the fourth postoperative day, with death between the third and eighth postoperative day. The most striking feature which led to the belated recovery of hemolytic staphylococcus in pure culture in one of our cases was the presence of exuberant masses of staphylococci in the shed watery exudate within the lumen and in the loose membranes covering the inflamed mucosa. In histologic sections, superficial necrosing effects near the masses of staphylococci were observed in a few cases. Other findings included very marked cloudy swelling of the kidneys, acute degenerative changes in the adrenal glands, recent focal necrosis in the liver, and acute staphylococcic bronchiolitic changes in the lungs. There was no evidence of septicemia.

These findings support the clinical and bacteriologic observations of Jackson, Finland, and co-workers in patients treated for pneumonia with terramycin. In 4 such patients the severe "superinfection" with pathogenic staphylococci was responsible for, or contributed seriously to, the fatal outcome. They suffered from severe diarrhea and staphylococcus was recovered from the stool. Our findings furnish the thus far largely lacking pathologic-anatomical substrate to this newly emerging disease of staphylococcic enterocolitis. Some factors probably responsible for this drastic change in the intestinal bacterial flora were discussed.

MODIFICATIONS OF STRUCTURAL CHANGES IN EXPERIMENTAL BACTERIAL GLOMERULONEPHRITIS FOLLOWING INTRARENAL ARTERIAL ANTIBIOTIC THERAPY. Fred C. Collier (by invitation) and Robert E. Klein (by invitation), Bowman Gray School of Medicine, Winston-Salem, N.C.

Abstract. The unique vascular system of the kidney, the combination of tissues of different embryologic origin, and the technical facility of controlling both infection and therapy presented particular advantages, suggesting that this organ be employed in a study of the direct action of antibiotics on inflamed tissue. Accordingly, the morphologic changes induced by terramycin and penicillin were studied in rabbit kidneys previously inoculated with washed suspensions of coagulase positive *Staphylococcus aureus* of appropriate sensitivity. Under ether anesthesia, staphylococcal suspensions were inoculated into the right and left surgically exposed renal arteries of 86 rabbits. Twenty-four hours postoperatively, 40 rabbits each received 25,000 units of penicillin in 0.5 ml. of saline solution, and 46 rabbits each received 1.25 mg. of terramycin in 0.5 ml. of saline solution. All antibiotics were injected into the surgically more accessible left renal arteries. The right kidneys, therefore, served as the inflammatory response controls. The animals were sacrificed and necropsied 24 hours after the single injection of the antibiotic, and the kidneys were

immediately placed in fixative. From each kidney, sections were prepared with hematoxylin and eosin, Masson's trichrome, and periodic acid-Schiff's stains.

Diffuse exudative glomerulitis, less constant tubular degenerative changes, and focal interstitial collections of granulocytes were present in sections of the control kidneys. In the kidneys which had received the intra-arterial antibiotics, however, the glomerular changes were more striking. Fibrinous exudates were frequent in the peri-glomerular spaces, a feature not common in the controls. Most impressive, however, was the proliferative glomerulitis with thickening of the glomerular basement membranes observed in the periodic acid-Schiff's stains of the treated kidneys. In addition to the proliferation of glomerular epithelial and interstitial cells, these glomeruli presented more marked ischemia and hypercellularity than did those of the control kidneys. The glomerular alterations encountered in the sections from kidneys treated with intra-arterial terramycin were found to be more profound than in those similarly treated with penicillin. A variety of interpretations may be placed on the presence of proliferative glomerulitis in the treated kidneys. It is equally possible that the antibiotics employed exerted an injurious effect on the inflamed glomeruli or that the proliferative changes might be a result of acceleration of the reaction to injury.

MORPHOLOGIC CHANGES IN PENICILLIN-TREATED CASES OF OTITIS MEDIA. Paul B. Szanto and Melvin Tamari (by invitation), Cook County Hospital, Chicago, Ill.

Abstract. An essential element in chemotherapy is the quantitative and qualitative alteration of inflammatory host response. Otitis media appeared to be a suitable subject for demonstrating that antibiotics frequently change the morphologic character of the disease. Biopsy and surgical specimens were obtained from the mastoids of 20 patients suffering from otitis media. Half of the patients were on penicillin treatment; the untreated half served as control. The histologic findings and the clinical course of the penicillin-treated cases of otitis media were compared with untreated cases of about the same duration. The morphologic differences were as follows:

1. In the course of penicillin treatment the character of the inflammatory cells changed. The inflammatory reaction of untreated otitis media is characterized by the exudation of polymorphonuclear leukocytes; after penicillin treatment, the reaction is characterized by the appearance of lymphocytes, plasma cells, and histiocytes.

2. Penicillin-treated cases were characterized by marked proliferation of fibroblasts and formation of collagen. The resulting fibrosis led to narrowing of the lumina of the mastoid cells. This type of morphologic change has not been found in untreated cases with the exception of untreated otitis media caused by type III pneumococci. Otitis media treated with penicillin resembled morphologically the otitis media caused by pneumococci type III of the pre-antibiotic era.

THE INFLUENCE OF CHEMOTHERAPY ON LEPROSY AS A GRANULOMATOUS PROCESS. George L. Fite, National Institutes of Health, Bethesda, Md.

Abstract. The granulomatous lesions of leprosy afford a unique opportunity for the study of the effects of drug treatment upon the natural course of a chronic infectious disease. True granulomatous tissues are formed, ready of access by biopsy. The lesions persist for long periods, enabling direct comparison and estimation of the effects of therapy. The only chemotherapeutic agent to come under consideration in leprosy is diamino-diphenyl-sulfone, introduced by Faget 12 years ago in the form of a dextrose-sulfonate derivative, and substantially proved in its value in the many quarters of the world in which it is now employed. Although not curative in its effect, arrest of the process is observed in 90 per cent of the cases or more.

Histologically, it is interesting to observe that the granulomas regress no more rapidly and in no different manner than in spontaneous regression of the disease,

which is observed infrequently. The changes that take place resolve quite slowly, with simple disappearance of bacilli and atrophy of cells. There is no resultant fibrosis, though the framework of the granulomatous tissue may persist indefinitely. In some cases the vacuolated cells, laden with lipids, persist in the lesions long after bacilli have disappeared. At times the lipids may be left extracellularly within the interstices of the fibers of the corium. It is interesting that bacilli and lesions regress most readily near the surface, persisting longer in deeper tissues. Bacilli persist within "globi" long after they have vanished from simple cells, and disappear from nerve fibers only at later intervals. The age of the granuloma appears to be a factor, the younger, fresher lesion responding more readily. The whole process may be written in terms of standard tissue responses, to which the drug does nothing whatever. The chemotherapy favors and enhances the regressive tendencies present in any chronic infection, probably by preventing further development of the causative organism. There is little or no evidence histologically to suggest that it destroys the organisms.

FACTORS MODIFYING THE CORTISONE-INDUCED DEPRESSION OF INFLAMMATION AND REPAIR. Raffaele Lattes and (by invitation) Ralph Jesser, Karl Meyer, and Charles Ragan, College of Physicians and Surgeons, Columbia University, New York, N.Y.

Abstract. Cortisone acetate in relatively large dose will inhibit or depress the phenomena of inflammation and repair, following injuries of different nature. The actual mechanism of this inhibition is still a matter of speculation. We have suggested that under the effect of cortisone, the injured tissues fail to release chemical substances necessary for the initiation and completion of repair. We also suggested the probable mucopolysaccharide nature of these substances (Lattes, R., *et al. Am. J. Path.*, 1953, **29**, 1–19).

Further evidence in favor of this hypothesis is supplied by the results of two groups of experiments:

A. In normal animals the implantation of pledgets of oxidized cellulose (glucosidoglucuronic acid) is always followed by a marked histiocytic response. These cells are large and have a strongly basophilic cytoplasm with metachromatic granules. When this experiment is repeated in cortisone-treated rats, the same histiocytic response occurs both quantitatively and qualitatively. The cortisone-induced inhibition of wound healing, however, is not modified. It is possible that with oxidized cellulose (an acid polysaccharide) we are supplying to the injured tissues some of the unknown factors which regulate the various phases of inflammation and repair, but which do not become available to the tissues under the effect of cortisone.

B. If pledgets of sterile, ordinary cotton gauze are introduced in the subcutaneous tissue of a rat, they will elicit a strong foreign body reaction. By the sixth day the gauze is firmly adherent due to granulation tissue which surrounds the foreign body and grows in its meshes. If the animal is treated with cortisone, the gauze does not become adherent and even after 6 days it can easily be removed from the site of its implantation. Using the same technique, acetyl-glucosamine and two polymers of hyaluronic acid were injected daily into the implanted pledgets of gauze in cortisone-treated rats. In cortisone-treated rats in which the hyaluronic acid polymers were used, the gauze was moderately adherent when the animals were sacrificed at the sixth day.

In addition, infection occurred at the site of the foreign body implantation in a small number of animals treated as above. The bacterial flora varied. In these animals, which were cortisone-treated, the gauze was firmly adherent and surrounded by a thick zone of edematous hyperemic tissue containing pockets of fibrinopurulent exudate. Microscopically, inflamed granulation tissue was found.

These experiments show again that there probably are substances which may

modify locally the cortisone-induced inhibition of the reparative processes. Moderate reparative response was obtained by local injections of two polymers of hyaluronic acid. More marked response occurred when spontaneous infection supervened. It is probable that this was not the effect of a specific bacterial species, but rather either the result of tissue damage caused by different bacteria with the release of substances necessary for the initiation of the reparative process, or the effect of products of the metabolism of these microorganisms.

CHANGES IN LEUKOCYTIC STRUCTURE AND FUNCTION DUE TO ACTH, CORTISONE, AND HYDROCORTISONE IN ACUTE INFLAMMATION IN MAN. John W. Rebuck and Raymond C. Mellinger (by invitation), Henry Ford Hospital, Detroit, Mich.

Abstract. Changes elicited by systemically administered ACTH, by topical cortisone, and by topical hydrocortisone in the exudative cells in acute inflammation in man were compared with each other and with various controls. The epithelium was scraped away from a small area of skin after which a primary excitant (usually diphtheria toxoid) was applied and the lesion covered with a coverslip. Within an hour cells of the inflammatory exudate had migrated to the under surface of the coverslip. When such migration had been effected, the coverslip was removed and additional coverslips were placed over the same lesion at timed intervals. The preparations were air-dried and stained like blood smears. In this way a series of permanent, fixed preparations of *in vivo* samplings of the cellular exudates were obtained from controls, from patients prior and subsequent to systemic ACTH therapy, and in subjects with lesions to which topical cortisone or topical hydrocortisone subsequently had been applied. In 18 controls the cellular cycle of acute inflammation consisted of early migration of neutrophilic leukocytes with minor participation by monocytes and tissue histiocytes. At 8 to 12 hours lymphocytes were present in increasing numbers. At 12 to 14 hours there was neutrophilic degeneration and contrasting lymphocytic hypertrophy. From 14 to 24 hours lymphocytic hypertrophy eventuated in lymphocytogenous macrophage predominance, reinforced by histiocytes. Subsequent to ACTH therapy or topical application of cortisone there was marked depression of lymphocytic participation at the 8 to 12 hour period of inflammation and as a consequence of the hypertrophying lymphocytes at 12 to 14 hours. Retardation and incompleteness of the cellular sequences ran parallel to depression of phagocytosis which was effected both by inhibition of phagocytic ability of neutrophilic leukocytes and by depression of cells phagocytic or potentially so: lymphocytes, hypertrophying lymphocytes, and resultant histiocytes. Lymphocytic depression was observed also after topical hydrocortisone, although later.

"SPONTANEOUS" INFECTIONS AFTER THE ADMINISTRATION OF CORTISONE AND ACTH. William Antopol and (by invitation) Howard Quittner and Ivan Saphra, the Joseph and Helen Yeamans Levy Foundation, Beth Israel Hospital, New York, N.Y.

Abstract. Early in the cortisone era, it was reported that over 20 per cent of mice receiving large doses of cortisone succumbed to disseminated infections (Antopol, W. *Proc. Soc. Exper. Biol. & Med.*, 1950, **73**, 262–265) with inflammatory foci in the liver, kidney, heart, hibernating fat body, and muscle. Colonies of bacteria without inflammatory reaction were present. The tissue adjacent to the "abscesses" showed varying degrees of degeneration and, at times, necrosis. Thrombi were frequently present. If the animals survived, attempts at healing by calcification were sometimes found. In almost all of the mice showing the above changes the *Corynebacterium pseudotuberculosis murium* was cultured. *S. enteritidis* was occasionally cultured; infection by this organism was usually associated with widespread liver necrosis and often with thrombi in the glomerular capillaries. These findings were of great interest since this strain of mice was relatively immune to the corynebac-

terium infection and subcutaneous inoculation of this organism in cortisone-treated mice produced widespread dissemination. It was believed that cortisone first caused a change in the reaction pattern of the mouse and in this altered state of reactivity profound changes were produced by organisms which under ordinary circumstances were incapable of producing appreciable damage. On this basis it was suggested that species known to be resistant to certain bacteria and viruses could, under the influence of cortisone stress, become susceptible to disease caused by these organisms.

A collateral study was made of the effects of cortisone on antibiotics in rabbits immunized to one of the several "O" antigens of the Salmonella group. It was found that the administration of cortisone during the period of immunization inhibited antibody production only slightly or moderately. However, a single large dose of cortisone produced a rapid fall in antibody titer when administered 2 to 3 weeks after immunization was completed. This fall occurred within 24 hours after the administration of cortisone and indicated that the cortisone-infection relationship was due in part to a depression of antibody.

In view of these experimental findings the occurrence of spontaneous infections during the course of cortisone or ACTH therapy was not wholly unexpected. In the last few years we have seen 2 cases of Hodgkin's disease and 2 of myeloic leukemia in which the supervening infection played a rôle in causing death. In both cases of Hodgkin's disease, extensive infection caused by *Aspergillus niger* was found at necropsy; in one, localized to the lung; in the other, disseminated. One patient with leukemia died of lobar pneumonia, with gram-positive cocci found in macrophages of the lung. The other case of leukemia succumbed with *E. coli* sepsis and duodenal ulcer containing *Monilia albicans*. A note of caution is necessary before indicting cortisone or ACTH as the only provocative factor responsible for the infection. Since nitrogen mustard and x-rays have effects similar in many respects to those of cortisone and may also produce infections, the rôle of these agents as well as the rôle of antibiotics must be considered.

MODERN CHEMOTHERAPY OF INFECTIOUS DISEASES: IMPLICATIONS AND SIGNIFICANCE TO PATHOLOGY. William H. Feldman (referee *), Mayo Clinic, Rochester, Minn.

CYTOCHEMICAL STUDY OF THE ACTION OF TRIETHYLENE MELAMINE ON TRANSPLANTED EXPERIMENTAL TUMORS WITH SPECIAL REFERENCE TO NUCLEIC ACID METABOLISM. Robert C. Mellors and Kanematsu Sugiura (by invitation), Sloan-Kettering Institute, New York, N.Y.

Abstract. The nucleic acids of the cell are of two generic types: desoxyribose nucleic acid (DNA), an important constituent of nuclear chromatin and of chromosomes; and ribose nucleic acid (RNA), which is a prominent component of the cytoplasm and of the nucleolus. The action upon cells of the nitrogen mustards and related compounds, hereinafter designated NM, is generally attributed to interference with the synthesis of DNA. This view is based upon observations of arrested mitotic division figures and nuclear abnormalities in normal and neoplastic tissues exposed to NM and upon chemical analyses of embryonic tissues, which disclose that NM impairs the synthesis of DNA but not of RNA. The present investigation provides information concerning the nucleic acid metabolism of cancer cells, such as those of the transplanted mouse tumor, carcinoma 1025, the growth of which is inhibited by treatment of the host animal with triethylene melamine, designated TEM. A conspicuous cytologic feature under such treatment is the development of large nucleoli. It is shown by interference microscopy that the protein mass of the nucleolus is much greater in cancer cells with arrested mitosis than in those that are actively proliferating, and it is disclosed by ultraviolet micro-absorption analysis

* By special invitation of the Council.

that the nucleic acid mass of the nucleolus is also greatly increased. The nucleic acid of the enlarged nucleolus is principally of the RNA type, as indicated by its enzymatic digestion with ribonuclease but not with desoxyribonuclease. Thus, the synthesis of RNA and of protein in the nucleolus is greatly enhanced in the cancer cell at the time when mitotic division is inhibited by TEM. The relation of this finding to the inhibition of mitosis in other types of cancer cells by TEM and other chemotherapeutic agents is under investigation.

THE DEVELOPMENT OF DISSEMINATED VISCERAL MYCOSIS DURING THERAPY FOR ACUTE LEUKEMIA. John M. Craig and Sidney Farber, the Children's Medical Center, Boston, Mass.

Abstract. Among 175 cases of acute leukemia in children which have been necropsied since the inception of active treatment of this disease by anti-folic compounds, the triethyl melamines, or by cortisone and ACTH, 13 cases were found at necropsy to have a disseminated visceral infection by various fungi. Eleven of these were due to members of the Candida family, and one each of Mucor and Cryptococcus. All had received heavy and long-continued medication with antibiotics. Penicillin, aureomycin, and streptomycin were most frequently used. The mycotic infection was accompanied in half the cases by widespread evidence of bacterial sepsis, usually due to gram-negative organisms. In the majority of cases the peripheral white counts were at extremely low levels in the last few days of life. In the overwhelming majority the portal of entry to the blood stream was through the mucosa of the tongue, esophagus, or intestine. Though the mucosa of these areas is subject to direct injury by antifolic and nitrogen mustard derivatives, such injury did not appear to have a rôle in these cases. Over the same period, 9 cases of treated leukemia showed at necropsy mycotic involvement of the intestine and tongue or esophagus, while 22 cases had only lingual or esophageal lesions. Only a single instance of disseminated visceral mycosis has been encountered in a nonleukemic patient, and none in untreated leukemic children.

THE EFFECT OF AMINOPTERIN ON GUINEA-PIG TUBERCULOSIS. Robert W. Pritchard (by invitation) and Donald M. Hayes (by invitation), Bowman Gray School of Medicine, Winston-Salem, N.C.

Abstract. The folic acid antagonist, aminopterin, has been extensively used in the treatment of childhood leukemia, and in several other conditions. It is a potent depressor of the hemopoietic and reticulo-endothelial systems, and has toxic effects on several other tissues, the gastro-intestinal mucosa and hair follicles among them. These widespread effects suggested that it may have played a rôle in altering the histologic appearance of the lesions and in the pathogenesis of a necropsied instance of miliary tuberculosis in a leukemic child who had been treated with aminopterin. The problem was approached experimentally.

The effect of aminopterin in daily doses of 2 μg. per gm. of body weight subcutaneously was investigated in tuberculous guinea-pigs. A total of 57 animals was used, with additional drug and environmental controls. Litter mates, all of roughly 100 gm. initial weight, were distributed in three groups, each receiving a standardized inoculum of $H_{37}RV$ human tubercle bacilli subcutaneously. One group received aminopterin from the time of inoculation with tubercle bacilli. The second was started on aminopterin as the tuberculin skin test of individual pigs became positive. The third group was not treated. The animals were individually caged, fed a standard diet, and at weekly intervals were weighed and skin tested with O.T. All living animals were sacrificed at the end of 6 weeks. Complete necropsies were done, and organ counts of tubercles were made. Several effects of aminopterin were noted. The drug plus tuberculosis resulted in a higher mortality than that from either the tubercle bacilli or aminopterin alone. Skin test sensitivity to O.T. did not develop

or was markedly delayed in this group. The tubercles in the organs of the aminopterin-treated animals were larger and less discrete, showed minimal or absent caseation, fewer lymphocytes, and larger numbers of acid-fast bacilli when compared with the untreated animals. The animals treated with aminopterin after the appearance of tuberculin sensitivity showed a lesser degree of this difference. These findings suggest that aminopterin is capable of altering the bodily reaction to tubercle bacilli in guinea-pigs, and affords another experimental means of influencing the host-parasite relationship in infectious disease.

MORPHOLOGIC EFFECTS OF ADRENALECTOMY IN HUMAN CANCER. Paul E. Steiner and Eleanor M. Humphreys, University of Chicago, Chicago, Ill.

Abstract. The post-mortem changes observed in cancers and in other parts of the body after bilateral adrenalectomy were studied in 32 persons. In most cases the adrenalectomy was preceded or accompanied by gonadectomy, hormone therapy, or other procedures; it was followed by cortisone and other replacement therapy. Because adrenalectomy has now been used for only about 27 months, our necropsy material is heavily weighted by the cases showing poorest responses. No effects on the general economy of the body attributable to the adrenalectomy were regularly observed. The cancers were divided according to site into three groups (prostate, mammary gland in both sexes, and miscellaneous), and according to therapeutic status as (a) no response, (b) initial response followed by relapse, and (c) those in which death occurred and histologic examination was made during a remission. To date our necropsy material on patients with carcinoma of the breast is limited. The most striking morphologic changes seen in our material up to this time have been in carcinomas of the prostate. Although no tumor has completely disappeared, conspicuous retrogressive changes have sometimes been seen. They included cell degeneration, maturation, and metaplasia. The degeneration may be rapid or slow, and consist of necrosis or atrophy through intermediate changes. The metaplasia was in the direction of a squamoid change. The cellular changes following adrenalectomy resembled more those of orchiectomy than of stilbestrol treatment. The prostatic cancers that escaped from control were of the usual, or of new, or of mixed types.

THE EFFECT OF CORTISONE AND COMPOUND F ON FIBROBLAST MIGRATION IN VITRO.* Nathan Kaufman, Earl J. Mason (by invitation), and Thomas D. Kinney, Cleveland City Hospital, Cleveland, Ohio.

Abstract. The effect of cortisone on embryo chick heart fibroblasts was studied, using a total of 1,798 explants. In addition, the effect of 17-hydroxycorticosterone was similarly studied, using 1,126 explants. Thus, a total of 2,924 explants was studied quantitatively. Under the conditions of this experiment the steroids cortisone and 17-hydroxycorticosterone had a definite inhibitory effect on the migration of fibroblasts in tissue culture. This effect increased with increasing concentration of the steroids used.

EFFECTS OF DEFICIENCIES OF CALORIES, PROTEIN, AND VITAMINS, INCLUDING FOLIC ACID (AMINOPTERIN), PANTOTHENIC ACID, AND RIBOFLAVIN ON THE MORPHOLOGY AND METABOLISM OF THE SMALL INTESTINE OF RATS. N. Zamcheck, J. J. Vitale, and D. M. Hegsted (all by invitation), Boston City Hospital and Harvard School of Public Health, Boston, Mass.

Abstract. Severe intestinal disturbances, including diarrhea, bleeding, ulceration, and perforation, may occur following the use of adrenal cortical hormones, antibiotics such as aureomycin or terramycin, and anti-carcinogenic agents such as aminopterin and related antimetabolites. Primary nutritional disorders such as pellagra and sprue are characterized by intestinal symptoms. Since the intestinal mucosa is known to

* This article will appear in a subsequent issue of *The American Journal of Pathology*.

be metabolically one of the most active body tissues, interference with its nutrition should be reflected promptly, functionally and morphologically. Accordingly, a systematic survey of the effects of nutritional deficiencies on the intestinal mucosa of rats has been initiated. Diarrhea and bleeding, occult or massive, were regularly observed in rats fed diets deficient in pantothenic acid or folic acid (aminopterin). The most marked pathologic changes were observed in animals injected with aminopterin and included petechial and massive hemorrhage, ulceration, and perforation of the small intestine. Some areas of epithelium appeared markedly damaged, while adjacent areas were spared. With large doses of aminopterin, the entire mucosa was obliterated by hemorrhage, inflammation, and degeneration. Less severe changes were observed in animals on pantothenic acid deficient diets. Deficiencies respectively of calories, protein, and riboflavin produced minimal changes. Simultaneous metabolic observations indicated reduction in oxygen uptake of duodenal mucosa of varying degree in all deficiencies. A variable lag between the earlier metabolic and the subsequent morphologic change was regularly observed. When tissue changes were marked, metabolic data could not be interpreted correctly without the aid of the morphologic findings. The necessity of combining morphologic and metabolic observations in such studies is apparent.

The results of these studies indicate that the intestinal mucosa reflects disturbances of nutrition promptly by functional and morphologic change. Such studies may aid in the understanding of the intestinal disorders associated with the use of potent chemotherapeutic agents.

THE EFFECTS OF BETA PELTATIN AND OTHER PODOPHYLLIN COMPONENTS ON THE TISSUES OF LABORATORY ANIMALS. Willard H. Eyestone (by invitation), National Cancer Institute, Bethesda, Md.

Abstract. The pathologic changes in the tissues of the mouse, rat, rabbit, and dog are essentially similar following the subcutaneous injection of the podophyllin components, podophyllotoxin, alpha peltatin, and beta peltatin. These changes fall into two categories, namely, (1) arrests in mitosis at the metaphase stage, and (2) cell necrosis. Depending upon the dosage of the drugs, their action may be demonstrated in cells within 15 to 30 minutes. The nuclear chromatin clumps or agglutinates into an irregular hyperchromatic mass and the cytoplasm appears swollen. Suggestive evidence is presented that some of these cells may return to normal. It is also indicated that some resting cells are vulnerable. The necrotic changes are characterized by karyorrhexis which may occur within 1 hour after the drug administration or by what appears to be a slower process with pyknosis, cytoplasmic eosinophilia and eventual cytolysis. Fatal doses caused severe depletion of the hematopoietic cells of the bone marrow. The cells most strikingly affected were the lymphocytes, thymocytes, myeloid cells, epithelium of the gastro-intestinal tract, and the germinal cells of the testis. Animals recovering from single or multiple injections showed no residual effects. Approximately 7 days are required for complete recovery in the mouse.

THE CYTOLOGY OF S-37 IN MICE TREATED WITH CHEMICAL AGENTS. Ross MacCardle (by invitation), National Cancer Institute, Bethesda, Md.

Abstract. An important problem relating to the effect of chemicals on protoplasm is to ascertain the cytologically detectable mechanism by which the chemical agent may injure a tumor cell and to determine whether such a mechanism may be characteristic of a particular chemical. At the National Cancer Institute, several investigators in Dr. Murray Shear's laboratory have surveyed in a preliminary way the capacity of a number of chemicals to produce necrosis and other changes in tumor cells at 8 to 48 hours following a single sublethal dose injected subcutaneously in the contralateral axilla of mice bearing Sarcoma 37 implanted in the thigh muscles.

Sarcoma 37 cells treated 24 hours previously *in vivo* with a single dose of 30 μg.

per gm. of body weight of podophyllin reveal multipolar mitotic figures, and many cells arrested in metaphase. The chromosome number of such cells is usually doubled and sometimes more than tripled. Under phase contrast microscopy the mid-plate region consists of a seething area of bubbling vacuoles, the matrix of which, upon microincineration, was found to be rich in minerals, chiefly iron. The Golgi apparatus in such chemically treated tumor cells swells and finally becomes filled with fatty droplets that may be colored with Sudan III. The mitochondria first become vesiculated and then appear as globules. The Nadi reaction indicates that at 8 hours after treatment with podophyllin the tumor cell is very rich in oxidases which at 24 hours have diminished.

None of these cellular changes are peculiar to any particular chemical studied. For example, sodium azide causes marked changes in the mitochondria of tumor cells, and lack of oxygen arrests mitosis in metaphase. The same changes occur also in non-malignant tissues treated with various chemicals. The growth of the fetal organ of Corti of the basal coil of the cochlea is arrested after treatment with podophyllin and its cells arrested in mitosis. The effects of chemicals both on malignant and non-malignant cells seem to be governed by the dose. Small doses of a chemical may damage tumor cells or fetal cells irreparably, whereas considerably larger doses are necessary to cause the same damage in adult normal tissues.

STUDIES ON THE MECHANISM OF ACTION OF SOME CARCINOLYTIC AGENTS. Murray J. Shear (by invitation), National Cancer Institute, Bethesda, Md.

Abstract. Tools are now at hand for the development of rational approaches to experimental cancer chemotherapy (Shear, M. J. *J. Nat. Cancer Inst.*, 1951, **12**, 569–581). The findings of empirical approaches, both in laboratory animals and in patients, have exceeded the expectations generally entertained a decade ago. For example, when the screening program in this laboratory (Laboratory of Chemical Pharmacology) was terminated, about 500 compounds of many different chemical classes had been found capable of producing necrosis in Sarcoma 37 following a single dose. Clinically, an increasing number of chemicals have been shown to exercise a favorable effect on the course of malignant disease of one type or another. The results obtained by many workers in various parts of the world have encouraged an expansion of effort in this field. Empirical work may yield drugs of greater efficacy and lower toxicity. Reliance, however, should not rest exclusively on this type of approach. Information is needed on the mode of action of these agents. It is not known how the potent drugs affect some tumors, why closely related compounds are negative, why other types of tumors fail to respond, why sensitive tumors become resistant, or what constitutes the damage to normal structure and function that is labelled "toxicity."

Various lines of attack on mechanisms of action, now in progress in this laboratory, were outlined and preliminary findings given. These include some of the effects in animals of the peltatins (isolated from podophyllin), of bacterial polysaccharides, and of other agents, upon such phenomena as oxidative enzymes, cellular biology of ascites tumors, rôle of the adrenal glands, etc.

CONSIDERATIONS IN THE INTERPRETATION OF MORPHOLOGIC CHANGES OCCURRING AFTER THE ADMINISTRATION OF THERAPEUTIC AGENTS. William Antopol, the Joseph and Helen Yeamans Levy Foundation, Beth Israel Hospital, New York, N.Y.

Abstract. Characterization of morphologic changes which occur after the administration of even a single substance is not simple. The specific primary changes produced by a compound must be differentiated not only from the changes secondary to "shock," inanition, supervening infection, or healing effects, but also from those alterations due to temporary or permanent inhibition or stimulation of endocrine or

other visceral functions, such as the hypoactivity of the pituitary, thyroid, and adrenal glands after the administration of larger doses of cortisone and the hyperactivity of these organs after the administration of sulfapyridine. Since the effects on various tissues may not be of the same order, disproportionate growth of tissues may occur. Thus Wolbach has shown that in hypervitaminosis A there is an acceleration of all processes of bone growth only. In the case of substances which are also produced physiologically, such as cortisone and ACTH, it is also necessary to distinguish those changes dependent upon physiologic mechanisms from those resulting from pharmacotherapeutic and toxic effects. Physiologically produced substances may also cause compensatory atrophy of those cells which are concerned with the production not only of the administered substance, but also of other vital compounds, thus producing deficiencies of all substances formed by such cells except that substance which is administered. Disturbance in adaptive balance mechanisms may also play a rôle.

In the pregnant animal, since there is considerable alteration both structurally and functionally of various vital organs, effects of administered compounds may be very different in some respects than in the non-pregnant animal. Thus the mammary glands in the pregnant animal show exaggerated enlargement after the administration of cortisone. Not only must consideration be given to the factors discussed above, but also to the effect on the fetus through stimulation and on the nursling through the milk, as well as to indirect effects on the fetus because of derangements, particularly endocrinologic, in the mother. In the mouse following the administration of large doses of cortisone 7 to 8 days before parturition, the fetuses die *in utero*, showing signs of retarded development and often autolysis; if given later in pregnancy, the babies were born alive, but died within 2 to 5 days. Fraser and Faunstat produced congenital malformations with cortisone. In our laboratories, Dr. Glaubach has shown that administration of cortisone to mother mice 9 to 12 days after parturition retarded the growth of the offspring, produced sparseness of hair, and the male offspring failed to show the expected growth rate until 40 days of age (normally the male grows faster than the female from the 28th day of age).

In general, in studying the effect of drugs, attention must be paid to the dose, the route of administration, the rate of absorption, and the vehicle in which the compound is dissolved or suspended. In addition the permanency or reversibility of the changes must be considered. Of great importance is the diet as well as the handling of animals, since stress of excitement causes changes which can influence the result. This is evident in the altered peripheral blood picture in the excited animal which may resemble the changes produced by cortisone.

CHEMOTHERAPY OF CANCER: IMPLICATIONS AND SIGNIFICANCE TO PATHOLOGY. Sidney Farber (referee *), the Children's Medical Center, Boston, Mass.

Abstract. Research emanating from several disciplines has been responsible for the discovery of the carcinolytic or carcinostatic action of a number of unrelated chemical compounds not only in the transplanted tumors in the mouse but also in disseminated cancer in man. These chemicals have been responsible for regression and even disappearance of disseminated tumors in man for months and even years. Such progress raises questions of importance in pathology, some concerned with the direct purpose of this research and others arising from the opportunity to use carcinolytic agents as new tools in experimental pathology. Questions of significance in pathology are:

1. The mechanism of action of these chemical compounds;

2. The necessity for detailed and accurate study of the life history and biological behavior of those tumors affected by chemical action and the altered vulnerability of these tumors to physical agents such as x-ray;

* By special invitation of the Council.

3. The recognition and nature of the toxic effects on normal tissues;
4. The development of methods for the rapid and accurate discovery of carcinolytic agents;
5. The effect of these chemicals on the mechanism of immunity to infectious disease;
6. The conditions under which carcinolytic agents may have carcinogenic potentialities; and most pressing of all;
7. The nature of resistance of cancer cells to chemical agents after an initial favorable response.

The techniques of the pathologist are indispensable not only for the guidance of the clinician in this new field of therapy but also for the acceleration of progress in basic research concerned with cancer chemotherapy. The biological activity of these new agents gives to the experimental pathologist a simple and effective tool in many fields such as antenatal pathology and the regular production of congenital malformations of precise nature.

LEUKEMIA IN GUINEA-PIGS. Charles C. Congdon and Egon Lorenz (by invitation), National Cancer Institute, Bethesda, Md., and Argonne National Laboratory, Chicago, Ill.

Abstract. Lymphatic leukemia has rarely been described in guinea-pigs. Three cases have been observed in 44 animals kept as controls and necropsied at 25 months of age or over. Five other cases have been found in 154 guinea-pigs necropsied at 25 months of age or over, which had received acute total body x-irradiation or chronic total body gamma irradiation. The neoplasm occurred with about equal frequency in non-inbred and two inbred strains of guinea-pigs. All control and experimental animals were under observation from 2 to 3 months of age until death, and periodic peripheral blood studies were made. The animals which developed leukemia showed an increase in lymphocytes or an increased leukocyte count or both. This increase appeared a few days to several weeks before the animals died or were killed. At necropsy the animals characteristically showed enlargement of lymphatic tissue, including lymph nodes, spleen, and Peyer's patches. There was microscopic infiltration of these organs. The liver was regularly involved. Bone marrow infiltration was variable in amount. Blood stream invasion was present in all animals. Many organs showed perivascular leukemic infiltration. In a few animals a pyelonephritic scar or pulmonary inflammatory area appeared to be a site of predilection for leukemic infiltration.

Transplantation lines were established from 4 primary leukemias occurring in inbred animals. One line was lost after going through 4 passages; one is now in its 17th and another in the 22nd passage. The 4th leukemia has just recently been transplanted from the primary host. These lines show different degrees of localization. One forms a large subcutaneous tumor at the site of transplantation and widespread leukemic infiltration, another shows rapid generalization from the transplant site without local tumor formation. In general, guinea-pigs with transplanted leukemia showed the same morphologic features of the disease that were seen in the primary cases.

Total body x-irradiation can be given at high dose levels up to 2000 r. and survival made possible by bone marrow injection. This treatment has produced temporary remission of the leukemic process in animals carrying one of the transplant lines.

TUMORS IN RATS FOLLOWING WHOLE BODY RADIATION. Simon Koletsky and G. E. Gustafson (by invitation), Western Reserve University Medical School, Cleveland, Ohio.

Abstract. A series of rats, each of which survived a single dose of 660 r. of whole

body radiation (approximately an LD_{70} dose), was followed for the development of neoplasms. Of 104 animals which survived for more than 6 months, 49 had one or more neoplasms, an incidence of 47 per cent. The mean time of death was 15½ months. A wide variety of both benign and malignant tumors was found, especially in skin, but also in connective tissue and viscera. Thirty of the 49 rats had malignant neoplasms, about evenly divided between sarcoma and carcinoma. The results indicate that whole body radiation is a potent carcinogenic agent.

THE INITIATION OF PULMONARY ADENOMAS IN MOUSE LUNG TISSUE BY BRIEF IN VITRO EXPOSURE TO A NITROGEN MUSTARD. Stanfield Rogers (by invitation), Duke University Medical School, Durham, N.C.

Abstract. The highly mutagenic nitrogen mustards have been shown *in vivo* to be carcinogenic for mice in several laboratories. The tumors thereby induced are of diverse sorts and varied degrees of malignancy. Pulmonary adenomas are among those more regularly appearing. As these mustards are known to be extraordinarily reactive and highly labile, decomposing in a few hours even *in vitro* in salt solution, it seemed important to find whether the carcinogenic activity was direct or through intermediary substances and if direct, to use the *in vitro* system of exposure to learn more of the nature and mechanism of the neoplastic change.

Lung tissue from embryo mice of the "C" strain nearing the last phase of gestation was procured by the method of Smith and Rous. The pooled lungs were finely hashed in Ringer's solution and washed until pale; half of the tissue was then put in a freshly prepared solution of nitrogen mustard in Ringer's solution and half in Ringer's solution alone. After an interval the hashings were separated from the solutions to which they had been exposed and thoroughly washed in Ringer's; they were then suspended in equal volumes of Ringer's and implanted in the posterior thigh muscles of adult mice of the same strain, the materials compared being implanted in opposite legs of the same animals. The mustard used was methyl-bis (beta-chloroethyl) amine hydrochloride (Merck). The concentrations studied ranged from 1-50,000 to 1-5,000,000. The maximum concentrations of mustard theoretically remaining after washing were 1-50,000,000 for the most concentrated and 1-5,000,000,000 for the most dilute solution tested. The interval of *in vitro* exposure ranged from 15 to 60 minutes. An interval of 10 weeks after implantation of the lung tissue was allowed for any tumors induced to become manifest. The implants were examined microscopically in serial section.

A 15 minute exposure of the lung tissue *in vitro* to a 1-5,000,000 solution of nitrogen mustard was found sufficient for the induction of pulmonary adenomas. No tumors were found in any unexposed implants. No tumors other than adenomas appeared in the time interval allowed. No growth of the implanted lung occurred following exposure to a solution of greater strength than 1-500,000. At this concentration the growth of the exposed tissue was considerably diminished as compared to that of unexposed tissue. This clear-cut effect on growth of the tissue spoke for rapid and complete penetration of the carcinogen throughout the hashed material. In the range of concentrations studied in which there was survival of the tissue there were no quantitative differences in the yield of adenomas. Thirty minutes was the optimum time of exposure to nitrogen mustard at 1-5,000,000, many more tumors being induced in such material than in hashings from the same mice exposed for either 15 or, curiously, for 60 minutes.

Only a few of the cells of the sort from which these adenomas arise underwent neoplastic change. Since penetration of the drug was evidently complete, it is clear that the susceptibility of the precursor cells to change must be conditioned by the presence or absence of some special cell state, possibly transient, but existent at the time of exposure to the carcinogen. As the lethal effects of the mustard on the lung

tissue are dependent upon concentration and over the same range the adenomatous response is not, it appears that the two effects are operating through different mechanisms.

STUDIES ON CARCINOGEN-INDUCED CARCINOMA OF THE CERVIX IN MICE. E. D. Murphy (by invitation), National Cancer Institute, Bethesda, Md.

Abstract. Two techniques have been devised for the application of 20-methylcholanthrene to the cervix of the mouse: (1) cervical painting: the cervix is visualized by means of an infant-sized otic speculum and painted with a saturated solution of methylcholanthrene in acetone; (2) endocervical thread: a silk thread impregnated with crystalline methylcholanthrene is suspended in the endocervical canal. The yield of the painting experiments was 13 squamous cell carcinomas of the cervix and vagina in 28 strain A mice with an average latent period of 9½ months, and 14 carcinomas in 46 C_{57} black mice, with an average latent period of 7 months. The yield with the thread technique was increased to 27 squamous cell carcinomas of the cervix in 35 strain A mice, and the average latent period shortened to 4½ months.

The tumors are predominantly well differentiated, and invade the rectum, bladder and parametrial structures, with late hydronephrosis. Although blood vessel invasion is readily demonstrated, there were no gross pulmonary metastases. Tumor emboli in the vessels of the alveolar septa were demonstrated in 4 cases. There were no lymph node metastases. Histochemical studies for mucin and glycogen revealed that the tumors tend to retain some of the peculiar metabolic functions of the epithelium of origin, and that these functions can be influenced by hormones. In a transplanted cervical carcinoma studied in intact females, intact males, castrate females, and castrate females bearing stilbestrol pellets, it was demonstrated that the degree of completeness of keratin formation in the tumor was dependent on estrogen.

Vaginal smears were taken during the course of carcinogenesis and criteria established for the diagnosis of suspicious and positive smears in the mouse. The earliest changes appear to be in superficial cells.

Several lesions analogous to carcinoma *in situ* were observed.

HOMOLOGOUS AND HETEROLOGOUS IMMUNITY AGAINST RAT LYMPHOSARCOMA CELLS. H. C. Stoerk, T. V. Budzilovich (by invitation), and T. C. Bielinski (by invitation), Merck Institute for Therapeutic Research, Rahway, N.J.

Abstract. Measurable immune activity against rat lymphosarcoma cells has previously been demonstrated in sera and in suspensions of lymphoid cells of rats in which lymphosarcoma transplants had regressed. This immune action has now been compared to that of sera and of lymphoid cells of rabbits intensely immunized with rat lymphosarcoma cells. *In vitro,* the homologous immune serum did not react in any measurable way with viable lymphosarcoma or other rat cells. Even after prolonged incubation of lymphosarcoma cells with homologous immune sera, the cells, when separated from the serum and injected into susceptible rats, proved viable and the sera exhibited no detectable loss of immune activity. The heterologous serum, following thorough absorption with rat red blood cells, agglutinated rat lymphosarcoma cells as well as normal rat lymphoid cells and gave precipitins with extracts of these cells. Homologous—but not heterologous—serum conferred passive immunity to susceptible rats. Viable—but not injured— lymphoid cells of immune rats, when mixed with lymphosarcoma cells, inhibited the growth of the latter in susceptible rats. This effect was observed with lymphoid cells from thymus as well as with those from other sources. Both viable and injured lymphoid cells from spleen or lymph node—but not from thymus of immunized rabbits—were cytotoxic to rat lymphosarcoma cells. Homologous immune sera, unlike heterologous immune sera and unlike the "homologous" immune sera of one highly inbred strain of mouse against

tumors of another strain (Gorer), could not be absorbed with rat erythrocytes nor with viable normal lymphoid or lymphosarcoma cells, but, as noted previously, lost their activity when they were exposed to extracts from a variety of animal tissues, including those of autologous origin.

THE CYTOTOXIC ACTION OF FRESH HETEROLOGOUS SERUM.* R. B. Stebbins (by invitation), J. A. Duddy (by invitation), and H. C. Stoerk, Merck Institute for Therapeutic Research, Rahway, N.J.

Abstract. Freund and Kaminer reported some 40 years ago that normal human serum—but not that of patients with cancer—was capable of lysing suspensions of carcinoma cells. Subsequently, Kraus and Graff observed that sera of rabbits and guinea-pigs, but not of rats or goats, contain a principle lytic for carcinoma cells. During a comparative study of homologous and of heterologous immune sera directed against rat lymphosarcoma cells, it was observed that lymphosarcoma cells, following exposure to fresh serum of a variety of animal species, failed to grow in susceptible rats. Loss of viability of the tumor cells was paralleled by distinct morphologic changes and by a loss of the ability of the cells to resist permeation by dyes like safranin. By the latter criterion, with few exceptions, fresh heterologous sera proved cytotoxic not only to rat lymphosarcoma cells, but also to normal lymphoid cells. Lymphoid cells from a variety of other species also were injured following exposure to certain fresh heterologous sera. The cytotoxic action of such sera was lost after heating at 56° C. for 30 minutes, at 37° C. after 1 hour, and during the course of several hours at room temperature. The addition of guinea-pig complement did not restore the activity of such sera. Normal human serum as well as that of 8 patients with extensive neoplastic disease proved to be cytotoxic to rat lymphosarcoma cells. Among some twenty species examined thus far, it appeared that the serum of each proved cytotoxic for lymphoid cells of every other species except in cases of such close relationship as that of rat to mouse or chick to pigeon.

MICROSPECTROPHOTOMETRIC DETERMINATIONS OF DESOXYRIBOSE NUCLEIC ACID (DNA) IN INDIVIDUAL CELLS OF NORMAL AND MALIGNANT HUMAN TISSUES.† Cecilie Leuchtenberger (by invitation) and Herbert Z. Lund, Institute of Pathology, Western Reserve University, Cleveland, Ohio.

Abstract. Since disparity of cell and nuclear size as well as hyperchromasia are among the criteria employed in the diagnosis of malignancy, it has been more or less assumed that tumor cells have an increased amount of DNA. The present report, which is a small part of an extensive study now in progress (C. Leuchtenberger and R. Leuchtenberger), is concerned with the quantitative estimation of DNA in normal human tissues, precancerous lesions (senile keratosis), and malignant tumors. Microspectrophotometric studies of DNA in normal human tissues of different individuals showed a constant average amount of DNA for the diploid cells of various tissues. In addition, some normal tissues (*e.g.*, liver) contain cells with multiple values of the diploid DNA content. The occurrence of DNA classes (1:2:4) in normal human cells parallels previous findings in normal animal tissues (Ris and Mirsky, 1949; Leuchtenberger *et al.*, 1951, 1952). Comparative studies of the DNA content in human cells of precancerous (senile keratosis) and cancerous lesions reveal DNA classes similar to that of normal cells, but show also cells with intermediate DNA content. Since, however, intermediate DNA values are equally present in normal tissues with mitotic activity, the variability of the DNA content in tumors cannot be regarded as a characteristic feature of malignancy.

* This paper was not presented because of illness.

† This investigation was supported (in part) by a research grant (C-1407) from the National Institutes of Health, Public Health Service.

ORIGIN AND DEVELOPMENT OF HETEROLOGOUS TUMORS OF THE UTERUS AND VAGINA. Robert P. Morehead, Bowman Gray School of Medicine, Winston-Salem, N.C.

Abstract. Heterologous mesodermal tumors arise in the uterus and vagina from cells of mesodermal origin which display both unipotential and pluripotential developmental capabilities. Occasionally heterotopic neoplasms are encountered which are composed of cells of only one type. It appears that these tumors arise from cells normally present in the uterus, but cells which display a latent potentiality of proliferation and differentiation in one direction. Pure lipomas and primary granulosa cell tumors and dysgerminomas of the uterus are illustrative of tumors of this type. Examples of neoplasms of this type were presented.

Heterologous mesodermal tumors of the uterus and vagina are more frequently of the mixed variety. It appears that these tumors arise from cells normally present in the uterus, but which possess a latent potentiality of proliferation and differentiation in more than one direction. Unfortunately, the widely adopted term "mixed mesodermal tumors" has become synonymous with a group of highly malignant tumors of the uterus. Large groups of both benign and malignant mixed mesodermal tumors of the uterus and vagina were presented and their origin and development discussed.

CARCINOMA OF THE PERIANAL DUCTS. Henry T. Grinvalsky (by invitation) and Elson B. Helwig, Armed Forces Institute of Pathology, Washington, D.C.

Abstract. The anal ducts and their relationship to diseases of the anal canal have received limited attention. Evidence has accumulated implicating the anal ducts in the development of ischiorectal abscess and fistula in ano, but these ducts have rarely been noted as the site of carcinoma. A review of the cases of carcinoma of the anorectal junction at the A.F.I.P. produced evidence which indicated that the probable site of origin of many of these carcinomas is the anal ducts.

In 26 patients the tumors arose in the region of the anorectal junction. Grossly they appeared as polypoid broad-based masses extending into the lumen of the bowel, or more commonly they grew beneath the anal and rectal mucosa and extended into the wall of the bowel, with or without surface ulceration. Follow-up studies of these patients reveal that 14 are dead from 2 to 36 months after operation. Necropsy was performed in 6 of this group of cases; there was no evidence of recurrence in 1, local recurrence in 2, and local recurrence, lymph node and visceral metastases in 3. Four patients were alive without evidence of recurrence 16 to 28 months postoperatively. One patient was still alive 3 years after operation but recently has shown local recurrence and metastases. No follow-up information is available on the remaining 7 cases.

These lesions were initially interpreted variously as baso-squamous carcinoma, transitional carcinoma, and basal cell carcinoma with keratinization. The similarity to some basal cell carcinomas of the skin was so striking that the diagnosis of basal cell carcinoma might have been made, except that peripheral palisading about the tumor cell nests was absent and metastasis occurred. Because of the histologic resemblance of this tumor to basal cell carcinoma, there is reason to believe that cases previously reported in the literature as basal cell carcinoma of the anus may be similar to the carcinoma arising in anal ducts which we have described.

TUMORS OF SWEAT GLANDS: THEIR DIAGNOSTIC SIGNIFICANCE. Olive Gates and Shields Warren, New England Deaconess Hospital, Boston, Mass.

Abstract. Tumors of a wide variety of histologic structure arise in sweat glands. The majority of tumors arise from ducts, the basal cells playing a leading part. Abnormal secretory activity is a dominant feature which produces characteristic stromal or epithelial changes, the more extreme forms producing the so-called "mixed tumor" or the "cylindroma." While counterparts of these sweat gland

tumors arise from any gland of external secretion, the closest both in structure and morphogenesis are those arising from mucous-serous glands of the eyelid, the respiratory tract, oral cavity, esophagus, major salivary glands, and to a limited extent of the breast. Tumors of sweat glands have little clinical importance since they are exceedingly rare (540 sweat gland tumors were received in this laboratory in 30 years) and usually benign, but because of their removal and availability to the pathologist in early stages of growth they are invaluable for the information they give on morphogenesis of glandular tumors in general.

"Granular Cell Myoblastomas" of Unusual Locations (Bronchus, Breast, Chest Wall). Leo Lowbeer (by invitation), Hillcrest Memorial Hospital, Tulsa, Okla.

Abstract. Three examples of so-called granular cell myoblastomas were reported. One is the second reported case of this neoplasm to originate in a bronchus; the patient was a 24-year-old colored female whose clinical symptoms were wheezing, cough, and expectoration. Roentgenogram showed atelectasis of the left lung, and on bronchoscopy a tumor was found obstructing the left main bronchus. The lung was resected and a polypoid tumor found in the lumen of the main bronchus, originating from the bronchial wall and showing no infiltration of the lung. The tumor consisted of granular cells, often arranged in bands and syncytial acidophilic ribbons, suggesting muscle origin. Intimate relations of tumor cells to nerves, but particularly to smooth muscle fibers, were found. The second case was that of a mammary neoplasm in a 60-year-old white female, grossly resembling a scirrhous carcinoma. The microscopic structure was that of a typical, uniform, granular cell myoblastoma. The acidophilia of the tumor cells was striking. No relation to nerves, muscle, or mammary tissue was found. The third example was a large neoplasm of the chest wall of a 40-year-old white male, consisting of huge, polyhedral, acidophilic, granular, vacuolated but fatless cells, often arranged in syncytial multinucleated ribbons. The tumor blended with the pectoralis muscle. No relation to nerves was found. Structure, tinctorial features, and a suggestion of myofibrils indicated a myogenic origin. The tumor was therefore diagnosed as a pleomorphic granular cell myoblastoma rather than the recently described alveolar soft-part sarcoma to which it bore some resemblance.

Neurogenic, histiocytic, and fibroblastic histogenesis of these tumors was discussed briefly, but their reported histochemical features do not seem to constitute proof of neural, mesenchymal, or fibroblastic origin. Involvement of nerves may represent infiltration rather than neural origin, and is not more conspicuous than is involvement of muscle in lesions of the tongue interpreted as infiltration by some and as myogenesis by others. The morphologic and tinctorial appearance, absence of mitotic figures, and cultural studies still seem to point to a myogenic origin. One neoplasm of the cheek observed by us showed definite evidence of the myogenic origin of granular tumor cells. Smooth muscle cells are contemplated as the possible source of the neoplasm. Additional histochemical studies for content of potassium and other substances are suggested.

Minute Peripheral Pulmonary Tumors: Observations on Their Histogenesis.* John T. Prior, State University of New York, Medical Center at Syracuse University, Syracuse, N.Y.

Abstract. A series of 20 peripherally located pulmonary tumors which have been "incidental findings" in our necropsy and surgical material were reviewed. The tumors do not appear to be associated with any particular co-existing pulmonary lesion, although bronchiectasis has been present in about one fourth of the cases. The majority have appeared in women and the average age at the time of discovery has

* This article will appear in a subsequent issue of *The American Journal of Pathology.*

been 57 years. The foci of tumor which were not observed on gross inspection of the pulmonary tissue have been characteristically subpleural in location. The growth pattern was characterized by both circumscribed epithelial nests imbedded in dense fibromuscular stroma and also by intrabronchiolar polypoid masses. The individual cells are round or spindle-shaped with vesicular nuclei, scant cytoplasm, minimal anisocytosis and poikilocytosis, and mitotic figures have not been identified. There has been no evidence of metastatic lesions in any of the group.

There is a striking similarity in growth pattern between bronchial adenomas of the carcinoid type and the present series of minute tumors. The intrabronchiolar masses of tumor have frequently been observed to be contiguous with the tumor in the fibromuscular tissue, and the resemblance between the basic cell comprising these tumors and the so-called "basal cell" of the normal bronchiole is impressive. The majority of those who have studied these small pulmonary tumors consider them to be an early stage of oat cell carcinoma, but the present study of both the clinical and histologic features has suggested that the tumor is an adenoma of bronchiolar origin and consistently benign in nature.

READ BY TITLE

ANATOMICAL CHANGES PRODUCED WITH EXCESSIVE DOSES OF CORTISONE IN THE NORMAL MOUSE, THE PREGNANT MOUSE, THE FETUS, AND THE OFFSPRING. William Antopol, the Joseph and Helen Yeamans Levy Foundation, Beth Israel Hospital, New York, N.Y.

Abstract. The administration of massive doses of cortisone to mice produces a striking lymphopenia; loss in body weight; atrophy of the thymus, lymph nodes, and spleen; diminution in size of the adrenal cortex, salivary glands, pituitary body, testis, seminal vesicles, prostate, and hibernating fat bodies.

In our investigations on the effects of cortisone in mice attention has been focused on the morphologic changes occurring regularly after the administration of massive doses of cortisone, particularly on the endocrine glands (adrenal, pituitary, and thyroid), on the lymphoid and other organs (spleen, lymph nodes, thymus, liver, salivary glands, and hibernating fat bodies), and on the peripheral blood picture and bone marrow. The effects on the pregnant mouse and its offspring also were studied. The reticularis of the adrenal gland and, at times, the inner follicular zone showed marked hyperemia in the first 24 to 48 hours; this is followed by shrinkage of cells. On withdrawal of cortisone there was restitution of the normal appearance in approximately 1 week. The x-zone did not appear to be affected by cortisone. The pituitary body became small, the acidophilic cells shrank and lost their affinity for eosin, becoming neutrophilic or slightly basophilic. The changes were reversible after withdrawal of cortisone. The thyroid gland showed inactivity as evidenced by an abundance of deeply acidophilic colloid and flattening of the acinar cells. The most striking anatomical change was reduction in size of the spleen. The weight was reduced to at least one third of the normal. This shrinkage was surpassed only by that which occurs after x-ray, compound F, and gramicidin. In the first 6 hours after administration of cortisone, slight karyorrhexis was evident; later no evidence of cell destruction could be found. The lymphoid tissue shrank and the sinuses collapsed. A residual small sheath of lymphocytes always remained about the eccentric arterioles. The septa were prominent. Polymorphonuclear leukocytes and eosinophils were relatively increased in the pulp. Similar changes occurred in the lymph nodes and thymus. In the latter organ numerous large cuboidal macrophagic cells, often fat-containing, appeared at the periphery. The liver, except where there was a complicating infection, showed an increase in glycogen. The liver cell nuclei were single

and appeared resting. The desoxyribonucleic acid content of the liver diminished. A considerable diminution in size of the hibernating fat bodies occurred in 24 to 48 hours. The cells shrank and the finely vacuolated cytoplasm became diffusely eosinophilic. A single dose of 2.5 gm. of cortisone was followed by a prompt and prolonged depression of circulating lymphocytes and eosinophils in the peripheral blood. The bone marrow showed a pronounced increase in the myeloid-erythroid ratio. The bones became fragile.

Twenty-four to 48 hours after the administration of cortisone to pregnant or nursing mice, the breast tissue enlarged and in a few days almost completely encircled the body. Histologically it consisted of markedly distended glands filled with milky fluid. In the pregnant mouse, two injections of 2.5 mg. of cortisone given 7 to 8 days before parturition caused retarded development and death of the fetus. When administered in the last 3 to 5 days of pregnancy the babies were born dead or died shortly after birth; when given 2 days before parturition the babies were born alive, but died within 2 to 5 days. Frazer and Faunstat produced congenital anomalies with cortisone. Mothers receiving cortisone 4 to 6 days after parturition were unable to bring up the offspring; all babies died in 3 to 5 days. In our laboratories, Dr. Glaubach has shown also that administration of cortisone to mice 9 to 12 days after parturition retarded the growth of the offspring, produced sparseness of hair, and the male offspring failed to show the expected growth rate until 40 days of age (normally after the 28th day, the male grows faster than the female).

CLINICOPATHOLOGIC AND IN VITRO BIOCHEMICAL EFFECTS OF CANCER CHEMOTHERAPEUTIC AGENTS. Maurice M. Black and Francis D. Speer (by invitation), New York Medical College, and Flower and Fifth Avenue Hospitals, New York, N.Y.

Abstract. Measurements have been made of the *in vitro* dehydrogenase activity of human cancer tissues in the presence and absence of urethane and triethylene melamine, employed singly and in combination. In 25 of the 75 cases studied, information was available as to the clinical response to x-ray and/or chemotherapy and an attempt was made to correlate the *in vitro* measurements with the clinicopathologic findings and the effects of x-ray and chemotherapy. On the basis of the findings to date it appears that:

(a) There is a decided variability in the intensity of the *in vitro* dehydrogenase activity of cancer tissue slices. This variability has not been correlated with the microscopic appearance of the tumor or its clinical response to x-ray therapy or chemotherapy.

(b) The clinical response to x-ray therapy is more closely correlated with the urethane inhibition of the *in vitro* dehydrogenase activity of the tissue slice than with the site of origin or the microscopic appearance of the tumor.

(c) In the majority of cases the *in vitro* effects of TEM tend to parallel those of urethane. The findings to date are consistent with the possibility that such *in vitro* measurements might presage the clinical response to TEM and analogous agents.

(d) Combinations of urethane and TEM may produce greater *in vitro* inhibition of the dehydrogenase activity of cancer tissues than either of the agents employed singly, a finding paralleled by clinical observations.

The findings of the present investigation were considered in relation to the morphology and metabolism of tumor tissues and the effects of cancer therapeutic agents.

THROMBOSIS AND PANCREATIC CARCINOMA. Ira Gore, Salt Lake General Hospital, Salt Lake City, Utah.

Abstract. An explanation has been suggested for the curious relationship between

carcinomas of the pancreas and thrombosis. The key factor would appear to be the presence of functionally intact but morphologically disrupted glandular tissue as the tumor bed. The frequency with which obstructing neoplasms of the head of the gland lead to atrophy explains the lesser incidence of intravascular coagulation with that localization. Metastases within the pancreas from tumors of various origins affect the coagulative mechanism in an identical fashion thereby reaffirming the insignificance of the histologic type of pancreatic neoplasm.

The clotting disturbance stems from the release of trypsin from the disrupted glandular tissue of the tumor bed. The thromboplastic effects of trypsin ordinarily are neutralized by the presence of antitryptic substances within the serum. Their titer, as determined by measurement of plasma antithrombin, rises considerably in acute pancreatitis. However, a mechanism which is operative in an acute short-lived process may fail when the stress which has invoked it continues over a long period. Progressive debilitation which occurs with cancer increases the possibility of such failure; should it occur, the continued release of trypsin from the tumor bed would lead to the occurrence of intravascular coagulation. Naturally such a mechanism would merely supplement the circumstances which contribute to thrombosis in other disease states. Frequent involvement of the arterial side of the circulation, particularly the heart valves, seems to be the only anatomical respect in which the thrombotic process differs from the much commoner form of the affliction.

INTRACRANIAL METASTASIS FROM CARCINOMA OF THE LUNG. Béla Halpert, William S. Fields (by invitation), and Michael E. DeBakey (by invitation), Veterans Administration Hospital and Baylor University Medical School, Houston, Texas.

Abstract. This study was made to determine the frequency of metastatic involvement of the brain from carcinoma of the lung. The clinical histories and findings at necropsy were reviewed in 100 consecutive patients with carcinoma of the lung, in 92 of whom the brain was included in the post-mortem examination. All patients were male (85 white, 15 negro) and 76 per cent were in the sixth decade of life or older. Intracranial metastasis occurred in 30 of the 92 patients. Among these, 55 were squamous cell, 23 reserve cell, 6 columnar cell, and 8 mixed (columnar and squamous) cell carcinomas. Metastasis occurred in 19 of the squamous cell carcinomas, 8 of the reserve cell carcinomas, 2 of the columnar cell carcinomas, and one of the mixed (squamous and columnar) cell carcinomas. Thus the frequency of intracranial metastasis seemed to bear no relationship to cell type. Local extension with involvement of regional lymph nodes occurred in 83 of the 100 cases. Metastatic foci were encountered in the liver 43 times and in the suprarenal glands 37 times. Metastasis occurred more often in the brain than in the skeleton (29 times), the pancreas (19 times), the kidneys (18 times), or the spleen (10 times). A single focus in the brain was observed in 12 and multiple foci in the remaining 18 patients. Among the 30 patients with intracranial metastasis, 10 had clinical manifestations of central nervous system involvement as the presenting complaint.

HETEROTRANSPLANTATION OF HUMAN TUMORS INTO CORTISONE-TREATED RATS. Cornelia Hoch-Ligeti (by invitation) and Y. T. Hsu (by invitation), University of Virginia School of Medicine, Charlottesville, Va.

Abstract. Biopsy material from human tumors was transplanted into cortisone-treated young Wistar rats. The rats received 25 or 12.5 mg. of an aqueous suspension of cortisone acetate subcutaneously at 2 or 3 day intervals. The number of the injections prior to transplantation varied between one to four; the injections were continued after the transplantation of the tumor. Rats were killed 4 to 21 days after transplantation, as a rule, after 8 days. The skin and muscle around the transplant were examined and any nodules found were studied histologically. With the exception of one rat killed 4 days after transplantation, no inflammatory reaction

was found around the transplant. The survival of the transplanted tissue was judged by the size of the transplant, by the healthy occurrence of the tumor cells, and the presence of mitotic figures. No serial transplantation was attempted. Transplants into groups of rats not treated with cortisone were unsuccessful. Of 9 tumors transplanted into 28 cortisone-treated rats, positive results were obtained with 8 tumors in 14 rats. A carcinoma of the kidney, the only tumor which could not be transplanted, was heavily infected. None of the tumors grew in all rats. A transplant of a bronchogenic carcinoma investigated 21 days after transplantation showed marked fibrosis and only a few degenerating tumor cells. Two other transplants of the same tumor were found persistent in rats killed 8 days after the transplantation. In 2 rats with successful grafts, blood counts were made when the rat was killed. The total white cell count was 1,900 leukocytes with 10 per cent lymphocytes in one, and 1,900 leukocytes with 7 per cent lymphocytes in the other. The leukocyte count of normal rats is 12,000 to 15,000 with 65 to 75 per cent lymphocytes.

It is as yet impossible to state whether the survival of human tissue transplants in rats is directly connected with the disappearance of lymphocytes or whether a disturbance occurred in the whole cellular metabolism of the cortisone-treated animals. It was not attempted to establish the percentage of all tumors in which heterotransplantation might be successful or which kinds of tumor are more suitable for transplantation. The aim of this study was to show that cortisone changes the inner environment of an animal in such a way that its resistance to heterotransplantation is greatly diminished.

ANOMALOUS PULMONARY VENOUS DRAINAGE. Abraham T. Lu (by invitation), Children's Hospital, Los Angeles, Calif.

Abstract. A review of the post-mortem examinations at the Los Angeles Children's Hospital revealed 11 cases of anomalous pulmonary venous drainage. These were summarized from the embryologic, clinical, and pathologic points of view in order to re-emphasize the recognition of a remediable group of anomalies.

LIPOPLASTIC LYMPHADENOPATHY. Robert P. Morehead and Sarah McClure (by invitation), Bowman Gray School of Medicine, Winston-Salem, N.C.

Abstract. Associated with the aging process in man, there is a gradual replacement of the parenchyma of lymph nodes by fat. This process is usually not associated with an increase in the size of the lymph nodes. Examples of rather marked visible and palpable axillary lymphadenopathy have been observed in which the increment in size of the nodes resulted from an excessive production of fat within these structures. The clinical entity was described and the origin and development of the lesion considered.

TISSUE LOCALIZATION RESPONSE TO RADIOACTIVE CHROMIC PHOSPHATE COLLOIDS. Lindon Seed (by invitation), James B. McCormick (by invitation), and George Milles, Augustana Hospital, Chicago, Ill.

Abstract. Colloidal chromic phosphate made with P^{32} has been injected locally and intravenously into normal animals. Studies include (1) chemical assay after various periods of radioactive decay; (2) radioactivity assay after various periods of decay; (3) study of histologic changes with varied concentrations of radioactivity and exposure; (4) histochemical studies; (5) localization of colloidal radioactive chromic phosphate after intravenous and local injection.

HYPOGLYCEMIA IN THE NEWBORN. A POTENTIAL BUT PREVENTABLE CAUSE OF DEATH. Joseph Tannenberg, the Genesee Laboratory, Batavia, N.Y.

Abstract. The post-mortem findings on an 11½ lb. baby born to a diabetic mother started the re-examination of the present widespread opinion that hypoglycemia in

the newborn is a harmless "physiologic" condition. Babies born to diabetic mothers, oversized babies, and premature babies have a greater mortality rate than average sized babies. The necropsy findings—evidence of infection, cerebral damage, pulmonary atelectasis, and the like—give satisfactory explanations only for certain cases. Even when difficult delivery, anesthesia, and faulty attempts at resuscitation are considered, there still remains a group in which death is not explained anatomically. These babies, which usually have survived 1 to 3 days, with increasing dyspnea in spite of an oxygen tent, generally show more or less complete pulmonary atelectasis without aspirated material. The lungs are markedly hyperemic and show more or less numerous and confluent petechial hemorrhages in the fine intralobular septa. These changes point to functional respiratory disturbances, most likely to a failure of the respiratory center.

The baby in question belonged to this group. It failed to breathe properly and was maintained in an oxygen tent until it died 7 hours after a spontaneous uneventful delivery from a diabetic mother. The thick panniculus adiposus and the hypertrophy of the Langerhans' islets were particularly impressive. That the latter might indicate increased insulin production and consequent hypoglycemia was first suggested by Dubreuil and his associates in 1920. This idea, however, had been generally rejected by subsequent authors for a variety of reasons which, in my opinion, do not stand critical evaluation. According to a review by McKittrick of the literature on blood sugar determinations in the newborn, its level is considerably lower than in the adult and its regulating mechanism is unstable in the first week, and particularly the first 3 days of life. Himwich and his associates have experimentally established the greater resistance of the newborn to anoxia than that of older children, and Wilson and his associates have shown that the ability of the brain of the newborn to split glucose anaerobically in addition to oxidizing it is the reason for the greater resistance to anoxia. Since, however, blood glucose is the sole source for the energy metabolism of the brain in the newborn and the adult alike, the importance of hypoglycemia as a cause of the unresponsiveness of the respiratory center becomes self-evident, particularly since the dangerous condition can easily be remedied by the injection of 10 ml. of 5 or 10 per cent glucose subcutaneously. The reason why blood sugar determinations are not more frequently done in the critical newborn period is seen in the relatively difficult procedure to obtain the necessary blood for even microdeterminations. As a tool to facilitate the collection of blood from babies for microdeterminations of glucose a special cup-pipette is offered that allows the measurement of single or multiple free-falling drops of blood that can be collected by pricking a toe or heel of the baby. A reprint of a patent paper illustrating the cup-pipette is available.

THE EFFECT OF STREPTOMYCIN ON THE GROSS AND HISTOLOGIC PICTURE OF TUBERCULOUS MENINGITIS. Kornel Terplan, Buffalo General Hospital, Buffalo, N.Y.

Abstract. The introduction of antibiotic treatment has added numerous new observations on the picture of tuberculous meningitis. The only thing which has changed is the duration of the disease, permitting a thus far unknown length of chronicity and recurrence of the inflammatory process. Original slides showing nontreated and treated cases of tuberculous meningitis demonstrated the pertinent distinctions.

In the classic picture of acute tuberculous meningitis, the space between pia and arachnoid is filled with fluid exudate rich in fibrin and leukocytes, sometimes of almost phlegmonous appearance, with more or less marked early caseation necrosis or necrobiotic changes. It surrounds arteries and veins uniformly and shows the most impressive changes in the small arteries and arterioles, with severe fibrinoid necrosis just as in the characteristic picture of allergic panarteritis. The walls of the veins, on the other hand, show dense cellular infiltration without necrosis. Some-

times recent thrombi are formed in veins, frequently leading to hemorrhages, from obstruction of venous return, in the corresponding parenchyma. In this stage, the extension of the meningitic process into the superficial cortex is already observed. In an occasional case the acute picture is even more impressive, with leukocytes almost exclusively present, which to some extent are undergoing rapid necrosis. There is, also, a considerable outpouring of macrophages, again with extensive necrosis. Only where caseation is more advanced, some attempts at formation of epithelioid tubercles are seen in these recent changes. There is, then, the absence of very characteristic tubercle formation and the presence of more non-specific phlegmonous inflammation with the caseation necrosis which is so characteristic in the picture of recent meningitis. Langhans' giant cells are not conspicuous. Another finding in the early stages is the formation of peri-adventitial tubercles, attached particularly to the veins, with masses of disintegrating macrophages resembling the typical Hortega cells seen in the brain itself. Complete occlusion of the small arteries and arterioles by endarteritic proliferation is not as frequent as in the chronic cases. Early formation of so-called meningocortical tuberculomas can be seen in the sulci in these early stages. These might be mistaken for primary tuberculomas of the cortex, discharging into the leptomeninges. Actual "pillow" formation, consisting only of subendothelial cells, is rare, or restricted to small segments in the blood vessels.

In the treated cases, huge meningeal tuberculomas were regularly found of a size which one rarely observes in non-treated cases. Here we see all the typical criteria of specific granulation tissue, with epithelioid giant cell tubercles. These large meningeal tuberculomas still contain caseated or necrobiotic centers. The secondary involvement of the parenchyma of the brain is, in many cases, less marked than one would expect. In smaller arteries there are very numerous endarteritic protrusions, sometimes occluding the lumen entirely. The cellular picture has changed considerably. Lymphocytes predominate; small meningeal tubercles have regressed into fibrous plaques. The changes in the brain substance secondary to interference with blood supply, though prominent in many of the treated cases, are in extent not more marked than in some of the non-treated cases. Huge tuberculomas in the tela and choroid plexus were seen only in the treated cases. In our cases in which streptomycin treatment was at least temporarily successful, we could not demonstrate original tuberculomas in the brain substance.

IMMUNE RESPONSE OF IRRADIATED CANCER PATIENTS TO VIBRIO METSCHNIKOVII VACCINE. E. Staten Wynne (by invitation), David B. Hinds (by invitation), Cora L. Gott (by invitation), W. O. Russell, and G. H. Fletcher (by invitation), University of Texas M.D. Anderson Hospital for Cancer Research, Houston, Texas.

Abstract. A series of three subcutaneous injections of a heat-killed *Vibrio metschnikovii* vaccine was given at weekly intervals to 29 patients undergoing x-ray or radium treatment for malignant neoplastic diseases, 13 non-irradiated patients with malignant neoplastic diseases, and 6 apparently healthy persons. Immune response was measured at intervals by determining the highest dilution of serum causing agglutinated growth of *V. metschnikovii* in an enriched broth medium. Therapeutic doses of x-irradiation ranged from 515 r. for the spleen or total body irradiation, to 20,850 r. for the sum of skin doses given to all portals, and dosages with radium ranged from 5,470 to 12,500 mgh. Intensive scattergraph analyses of the data obtained by the "growth agglutination" technique failed to reveal detectable differences in response in the three groups studied. However, since the production of bacterial agglutinins may not invariably parallel resistance to infection, it should not be concluded that the entire immunologic defense mechanism is necessarily unaffected by high doses of therapeutic irradiation.